Seasonal effects of drought on the productivity and fodder quality of temperate grassland species

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Knowing trees, I understand the meaning of patience,
Knowing grass, I can appreciate persistence.

Hal Borland

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Introduction

Longtime observations of the atmosphere and surface of the earth provide comprehensive evidence for climate change. Climate change is a term used to encapsulate alterations in the state of the earth's climate that are characterized by changes in the mean and/or the variability of its properties that persist for an extended period of time (Hartmann *et al.*, 2013). These changes comprise alterations in the hydrological cycle and atmospheric circulation, changes in the atmospheric composition, such as increases in greenhouse gases including carbon dioxide, methane and nitrous oxide, increases in mean surface temperature (0.72°C over the period 1951-2012 so far) and increases in the occurrence of extreme events, such as heat waves, heavy precipitation events or extended drought periods (Hartmann *et al.*, 2013).

Drought is the prolonged absence or deficiency of precipitation that results in water shortage (Pachauri *et al.*, 2015), but definitions are diverse (Trenberth *et al.*, 2013). The important characterization of drought, however, is based on the role of climatic factors such as precipitation, evapotranspiration (ET) and runoff. Drought events occur naturally and are not manufactured by climate change, but under climate change the increased surface temperature leads to an increase in ET resulting in an increased rate of drying (Trenberth *et al.*, 2013). This means that drought events under climate change are established more quickly, with a higher intensity and are probable to last longer (Trenberth *et al.*, 2013). In the past, studies on climate change have proven an increase in drought events, but confidence on future development could be higher (Hartmann *et al.*, 2013). Since there is a lack of direct measurements of drought, such as soil moisture or soil water potential, drought is often assessed via proxies, which lead to lower levels of confidence of projections. Predictions of drought events that increase in frequency and intensity were described as likely (according to the definition by Hartmann *et al.* (2013) this equals an assessed likelihood from 66-100%) in 2013. However, the 2015 drought in Central Europe showed that future drying trends could become stronger than assumed from the last IPCC report (Orth *et al.*, 2016).

Although drought events are predicted to increase in the near and far future, not all areas are affected likewise, since predictions differ temporal and spatially (Orlowsky & Seneviratne, 2012; Greve *et al.*, 2014). Nonetheless, large parts of the world's terrestrial surface will most likely be subject not only to precipitation decreases, but also to increases in drought events, including southern Europe and the Mediterranean, the Middle East, Southern Africa and parts of the Southern US (Orlowsky & Seneviratne, 2012). For Switzerland, multi-model projections show seasonal differences in changes of precipitation. Especially in summer the expected precipitation is likely to decrease severely (Frei *et al.*, 2006) leading to an enhanced likelihood of multi-day dry spells (Fischer *et al.*, 2015).

Large parts of the terrestrial ecosystem consist of grasslands. More or less recently (c. 2003) grassland ecosystem occupied roughly ¼ of the world's land surface (Mason &

Zanner, 2005) and are distributed all over the world, since their distribution is primarily based on climatic factors that influence soil moisture availability (Mason & Zanner, 2005). However, temperate grasslands are an important carbon sink, they cover sites unusable for other purposes and are widely used for agriculture. In Switzerland, for example, more than 70% of the area used for agriculture consist of grasslands (Bundesamt für Statistik (BFS), 2018). Droughts reducing soil water availability will affect photosynthesis and growth of plants. Hence, ecosystem productivity and, moreover, a variety of ecosystem processes may be affected potentially transforming grasslands into carbon sources (Friedlingstein *et al.*, 2006).

In the past and with increasing interest, a multitude of studies have tried to assess the impact of droughts on ecosystems. Especially, impacts on the ecosystem productivity have been studied numerously (Wu *et al.*, 2011). Not surprisingly, in field experiments water limitation due to dry spells has shown to reduce productivity of ecosystems (Wu *et al.*, 2011). Nonetheless, quite large differences among the ecosystem responses to drought have been recognized (Gilgen & Buchmann, 2009; Hoover & Rogers, 2016). Ecosystem diversity, for example, has shown to increase the resistance to drought, reducing productivity losses of ecosystems with higher species richness under drought, above- and belowground (Kahmen *et al.*, 2005; Wagg *et al.*, 2017). Moreover, the plant functional composition of an ecosystem also increases resistance to drought regarding biomass. Hofer *et al.* (2016) showed that mixing grasses with legumes, for example, increases yield under drought when compared to the yield of the respective monocultures. On the other hand, higher land use intensity, generally, reduces the resistance under drought leading to lower biomass production (Vogel *et al.*, 2012; Zwicke *et al.*, 2013). Yet, a study by Walter *et al.* (2012) showed that more frequent mowing increased productivity in the first year as the result of overcompensation, although this effect vanished in the second year of the experiment. However, a problem that also impacts the reliability of climate change models for drought events, as above mentioned, is the use of a proper measure for drought. Without a common and reliable measure (such as soil water potential) the comparability and confidence of drought responses will remain difficult (Vicca *et al.*, 2012).

Alterations in the water cycle of temperate grasslands under drought, however, are affecting the productivity of plants due to ecophysiological changes, since photosynthesis is strongly linked to processes of the gas exchange. So far, studies that examined the effects of droughts on the water balance of plants have found strong differences between species in the gas exchange. Nonetheless, stomatal conductance rates (g_s) and leaf water potential (Y_{Leaf}) were generally reduced by drought (Jackson, 1974; Medrano *et al.*, 2002; Signarbieux & Feller, 2012; Bollig & Feller, 2014), consequently reducing yield (Turner & Jones, 1980).

As grasslands are widely used for agriculture (e.g. cattle farming), alterations of the nutrient status and the forage quality are of high importance for this sector. The nutrition of plant material is based on a variety of nutrient contents. Nitrogen (N), especially in the form of crude protein (CP) is a key nutrient for animal nutrition. Moreover, structural carbohydrates in the form of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) determine the quality of produced forage meaning that higher contents lead to a reduced digestibility. During plant maturation, the lignin concentration in the cell wall increases, which binds to structures such as hemicellulose making the plants less digestible for the rumen (Smith *et al.*, 1998). In general, digestibility not only reduces over the growing season as Schubiger *et al.* (1997) and Smith *et al.* (1998) have shown for several species, but also differ between species naturally (Grant *et al.*, 2014).

Based on varying climatic conditions in temperate regions and due to different developmental, morphological and physiological stages plants undergo, temperate grasslands show a distinct seasonality. While plants undergo different developmental stages throughout their life cycle and also differ in their physiological performance the productivity of grassland ecosystems is highly variable throughout the year. In general, grasslands show high rates of productivity early in the growing season and increases with expansive growth. After a first productivity peak early in the growing season, growth rates decline as anthesis sets in (Voisin, 1988).

The seasonality of grasslands with altering physiology and ontogeny throughout a year, thus, potentially leads to different sensitivities to droughts depending on when they occur in the growing season. Previously, higher sensitivities of reproductive stages to drought, for example, have been examined for several crop plants (Chinnici & Peterson, 1979; Fischer, 1979; O'Toole & Cruz, 1980). Also, evaporative demand throughout the year is changing. This leads to differences in evapotranspiration rates in the plants, affecting the plants' water status. Seasons with higher evaporative demand, thus, have the potential to reduce photosynthesis and growth rates. To date, studies examining the seasonality of drought events on temperate grasslands do not exist, although Swemmer *et al.* (2007) found that productivity in temperate grasslands strongly differed depending on the timing of precipitation events.

However, since climate change predictions project increases in extreme events for a variety of temperate regions, droughts are supposed to become more regular and also intense. Considering the known variability of grassland production throughout the year and the sensitivity of grasslands to droughts in general, it is of high importance to analyze and understand seasonal differences in the response of grasslands due to drought. Disentangling the effects of seasonality and drought in grasslands is inevitable for dynamic modelling of future global vegetation responses and carbon (C) balances. Additionally, rising interest in sustainable and simultaneously productive land use under climate change is not only concerning the agricultural sector, but society.

This thesis shall help providing a better understanding of the seasonal effects of drought events primarily on productivity, but also on underlying ecophysiological processes and the direct effect on ecosystem services grasslands provide. The thesis is structured into three chapters, each addressing one main topic of seasonal drought events in grassland.

In *Chapter 1* immediate effects of seasonal drought events on growth rates, as well as legacy effects that might occur in the post-drought period after drought-release are examined. Moreover, the impact on annual aboveground net primary productivity (ANPP) is investigated to test the influence of resistance and resilience in grasses and to examine the sensitivity of ANPP to seasonal droughts.

Chapter 2 concerns the different sensitivities of plants to droughts occurring in different seasons. We tested if differences in drought sensitivities are due to i) varying drought intensities, ii) depend on the plant developmental stage (PDS) or iii) if the drought experienced by the plants differs between the seasons.

Chapter 3 focusses on the forage quality under seasonal drought events. Here, alterations in a variety of forage quality parameters due to drought in general and also to seasonal drought period in particular are investigated.

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Chapter 1

Timing of drought in the growing season and strong legacy effects determine the annual productivity of temperate grasses in a changing climate

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Abstract

In a two-year field experiment we tested, if the resistance and resilience of grasses towards drought varies throughout a growing season and if the timing of drought, thus, has an influence on drought-induced reductions in annual ANPP of grasses. For the experiment, we grew six temperate perennial C3 grasses in a field as monocultures that were cut six times in the growing season. The grasses were subject to 10-week drought treatments that occurred either in the spring, in summer or in the fall. Across all species drought induced losses of productivity were smaller (-20% to -51%) than in summer and fall (-77% to -87%). This suggests a higher resistance to drought in spring when productivity of the grasses is the highest and plants are in their reproductive stage. After the release from drought we found no prolonged suppression of growth. In contrast, post-drought growth rates of formerly drought stressed swards outperformed the growth rates of the control swards. In 2014, the overcompensation after drought release was similar in all seasons, but differed in 2015. The strong overcompensation of growth after drought release resulted in relatively small overall drought induced losses of annual ANPP that ranged between -4% to -14% and were not affected by the timing of the drought event. Our results show that (i) the resistance of growth rates in grasses to drought varies across the season and is positively correlated with growth rates in the control, (ii) that positive legacy effects of drought indicate a high resilience of temperate grasses to drought, and (iii) that the high resilience can compensate immediate drought effects on total annual biomass production to a large extent.

Introduction

Temperate permanent grasslands cover 38% of the agricultural area of Europe and deliver essential ecosystem services (Suttie *et al.*, 2005; Pilgrim *et al.*, 2010). These services include the production of fodder for livestock and the dairy industry (Voigtländer & Boeker, 1987), the maintenance of biodiversity (Lachat *et al.*, 2010), and the sequestration of substantial amounts of carbon (Schulze *et al.*, 2009). Climate projections forecast significant rainfall reductions in summer for central Europe (Fischer *et al.*, 2015; CH2018, 2018). Such drought periods will influence physiological processes of ecosystems and consequently affect the ecosystem services that are delivered from permanent European grasslands (Reichstein *et al.*, 2013).

Numerous studies have attempted to quantify the effects of drought on grassland ecosystems in the past decade. In general, these studies have confirmed that drought-induced water limitation typically leads to a reduction of net primary productivity (NPP) (Wu *et al.*, 2011; Wilcox *et al.*, 2017; Gherardi & Sala, 2019). Importantly, however, these studies have also shown that the response of ecosystems to experimental drought can vary quite dramatically (Gilgen & Buchmann, 2009; Hoover *et al.*, 2014; Grant *et al.*, 2014; Wilcox *et al.*, 2017; Gherardi & Sala, 2019). Among others, the drought response of grasslands has been shown to depend on the severity of the experienced drought (Vicca *et al.*, 2012; Wilcox *et al.*, 2017), and important secondary factors such as the type of grassland affected (Byrne *et al.*, 2013; Sala *et al.*, 2015; Wilcox *et al.*, 2017; Gherardi & Sala, 2019), the intensity of land use (Walter *et al.*, 2012; Vogel *et al.*, 2012), the plant functional composition (Gherardi & Sala, 2015; Hofer *et al.*, 2016, 2017a; Mackie *et al.*, 2018), or the biodiversity of an ecosystem (Kahmen *et al.*, 2005; Isbell *et al.*, 2015; Wagg *et al.*, 2017). These secondary factors that affect the responses of terrestrial ecosystems to drought are just beginning to be understood (Wu *et al.*, 2011; Reichstein *et al.*, 2013). Defining their impact on the drought response of terrestrial ecosystems is yet essential for quantitative predictions of drought effects on the carbon (C) cycle and for the ultimate inclusion of drought responses of terrestrial ecosystems in coupled land surface models (Schiermeier, 2010; Smith *et al.*, 2014).

Grassland ecosystems often show a pronounced seasonality, where plants undergo different phenological, physiological, morphological or ontogenetic stages throughout a year (Voigtländer & Boeker, 1987; Gibson, 2009). Temperate European grasslands for example, are highly productive early in the growing season during reproductive growth, while they show much lower growth rates during vegetative stages in summer and fall (Voisin, 1988). Several studies have addressed how the seasonal timing of drought affects aboveground NPP (ANPP) of North American C4 grasslands (Nippert *et al.*, 2006; Petrie *et al.*, 2018). It has been suggested that moisture availability during stalk production of the dominant C4 grass species in mid-summer is particularly important for maintaining the annual productivity of these grasslands (La Pierre *et al.*, 2011; Denton *et al.*, 2017). For C3 dominated temperate grasslands, this would imply that spring, when grasses flower and have the highest growth rates, is the time when the productivity should be most susceptible to drought and that productivity should be less prone to drought-induced losses in the summer and fall. Empirical evidence how the seasonal timing of a drought event affects the productivity of temperate C3 dominated grasslands is, however, missing.

The impact of drought on the annual NPP of ecosystems depends on the immediate effects of drought on productivity (determined by the drought resistance of the ecosystem), but also on potential legacy effects that occur after drought release (determined by the drought resilience of the ecosystem) (Seastedt & Knapp, 1993; Sala *et al.*, 2012). In particular, legacy effects of drought are a critical yet rarely explored component that can strongly affect the impact of drought on the annual NPP of an ecosystem (Sala *et al.*, 2012; Ingrisch & Bahn, 2018; Petrie *et al.*, 2018). Previously it was believed that the drought history (e.g. previous year annual precipitation deficit) of an ecosystem is crucial for the annual NPP and that the magnitude of the drought history negatively influences current NPP (Yahdjian & Sala, 2006; Sala *et al.*, 2012; Reichmann *et al.*, 2013; Mackie *et al.*, 2018). In contrast, there is now increasing evidence that drought stressed plants or ecosystems can respond to drought release also with an overcompensation of their physiological activity or growth (Shen *et al.*, 2016; Hofer *et al.*, 2017a; Griffin-Nolan *et al.*, 2018). Following an experimental drought, tropical and temperate tree seedlings have, for example, exhibited higher net photosynthesis rates than seedlings that had not experienced a drought event (Hagedorn *et al.*, 2016; O'Brien *et al.*, 2017). In grasslands, Hofer *et al.* (2016) recently showed that formerly drought-stressed swards had a higher productivity in the post-drought period than non-stressed control swards and that the species richness of a grassland contributes to this effect (Kreyling *et al.*, 2017; Wagg *et al.*, 2017). Even across growing seasons it has been suggested that the previous growing season precipitation patterns can have positive legacy effects on the current year productivity of ecosystems (Shen *et al.*, 2016). As legacy effects can either worsen or diminish immediate drought effects on annual NPP, their assessment is essential to determine if the sensitivity of annual NPP to the timing of drought is driven by the resistance or resilience of the system (Shen *et al.*, 2016; Petrie *et al.*, 2018). This requires, however, a detailed analysis of not only annual NPP, but the assessment of biomass increase (i.e. productivity) during and after the release of a drought event.

In the work that we present here, we experimentally assessed if the drought response of the annual NPP of six different grasses that are common in temperate C3 grasslands depends on the timing of the drought event in the growing season. To do so, we determined the drought resistance and resilience for these grasses in different times of the growing season. Specifically, we tested in our study,

i. if the immediate reduction of aboveground productivity during drought – i.e. the resistance of an ecosystem – differs in different times of the growing season,
ii. if the direction and magnitude of legacy effects on aboveground productivity – i.e. the resilience of an ecosystem – differ in different times of the growing season, and
iii. how the combination of resistance and resilience in different times of the growing season impact the annual ANPP of drought-stressed temperate C3 grasses.

Methods

Research site

The experiment was performed in the years 2014 and 2015 near Zurich, Switzerland (47°26'N, 8°31'E, altitude: 490 m a.s.l., mean annual temperature: 9.4°C, mean annual precipitation: 1031 mm) on an eutric cambisol soil. For the experiment, we established six perennial C3 grasses in monoculture that are commonly used in agricultural practice in August 2013 on 168 plots (3 × 5 m). The plants were sown on a highly productive field that yields typically around 12 t grass dry matter per year and hectare (i.e. 1200 g/m^2). The establishment followed the basic procedures of sowing permanent highly productive grasslands, where before sowing the existing vegetation at the site (which was a winter wheat) was plowed. Establishment of the grasses in the growing season before the experiment started followed best practice and guaranteed full establishment of the swards (including vernalisation during winter) and full productivity in the following year. The six grasses were *Lolium perenne* L. early flowering (LPe; cultivar 'Artesia'), *Lolium perenne* L. late flowering (LPl; cultivar 'Elgon'), *Dactylis glomerata* L. early flowering (DGe; cultivar 'Barexcel'), *Dactylis glomerata* L. late flowering (DGl; cultivar 'Beluga'), *Lolium multiflorum* Lam. var *italicum* Beck (LM; cultivar 'Midas'), and *Poa pratensis* L. (PP; cultivar 'Lato'). Phosphorous, potassium and manganese were applied following national Swiss fertilization recommendations for intensely managed grasslands at the beginning of each growing season (39 kg P/ha, 228 kg K/ha, 35 kg Mg/ha). In addition, all plots received the same amount of mineral N fertilizer as ammonium-nitrate (280 kg N/ha, divided into six applications per year). The solid N fertilizer was applied at the beginning of the growing season (80 kg N/ha) and after each of the first five cuts (40 kg N/ha each time).

Experimental design

Each of the six grasses was subject to four treatments: one rain-fed control and three seasonal drought treatments (spring, summer, fall) (see Fig. 1). A drought treatment lasted for ten weeks. Drought was simulated using rainout shelters that excluded rainfall completely on the treatment plots. The rainout shelters were tunnel-shaped and consisted of steel frames (3 × 5.5 m, height: 140 cm) that were covered with transparent and UV radiation transmissible greenhouse foil (Lumisol clear, 200 my, Hortuna AG, Winikon, Switzerland). To allow air circulation, shelters were open on both opposing short ends and had ventilation openings of 35 cm height over the entire length at the top and the bottom at both long sides. These shelters had previously been successfully tested in other grassland-drought experiments (Hofer *et al.*, 2016, 2017a,b). Rain-fed controls were subject to the natural precipitation regime. However, when soil water potential (Ψ_{Soil}) sank below -0.5 MPa due to naturally dry conditions, control plots were additionally watered with 20 mm of water (300 l per plot). Watering happened once on June 16th and 17th 2014 and three times in 2015 (7.7., 14.7., 11.8.).

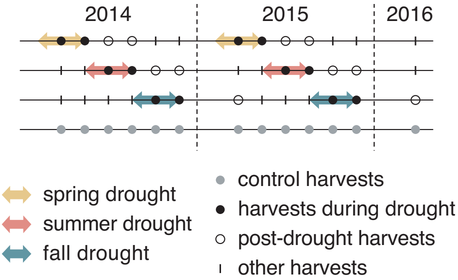

Fig. 1: Experimental design; experiment lasted two consecutive years (2014, 2015) with twelve harvests (•) evenly distributed over both growing seasons in each treatment and one additional harvest in the beginning of 2016. Arrows indicate the duration of each drought treatment (ten weeks). Each treatment was replicated four times.

Environmental measurements

Relative humidity and air temperature were measured hourly at the field site using VP-3 humidity, temperature and vapor pressure sensors (Decagon Devices, Inc., Pullman, WA, USA). Measurements were conducted in control and treatment plots under the rainout shelters (n=2). Information on precipitation and evapotranspiration was provided by the national meteorological service stations that were in close proximity of our research site (average of the two surrounding meteorological stations Zurich Affoltern in 1.4 km distance and Zurich Kloten in 4.5 km distance). Ψ_{Soil} was measured in 10 cm depth on an hourly basis using 32 MPS-2 dielectric water potential sensors (Decagon Devices, Inc., Pullman, WA, USA). The soil water potential sensors were evenly distributed over the field and treatments. Daily means of all measurements were calculated per treatment, but across grasses since no grass-specific alterations in Ψ_{Soil} were expected (Hoekstra *et al.*, 2014) or measured (n=8).

Harvests

Aboveground biomass was harvested six times per year in five-week intervals in 2014 and 2015, resulting in six growth periods per year (see Fig. 1). Aboveground biomass was also harvested once in spring 2016. Such a high frequency of harvests is typical for highly productive grasslands used for fodder production. For the purpose of our study this high-resolution biomass sampling allows the analyses of the immediate drought effects and the impacts of drought that occur after the release of drought on productivity. The harvests were synchronized with the drought treatments and occurred five and ten weeks after the installation of the shelters on a respective treatment. For the harvests, aboveground biomass was cut at 7 cm height above the ground and harvested from a central strip (5 × 1.5 m) of the plot using an experimental plot harvester (Hege 212, Wintersteiger AG, Ried/I., Austria). The fresh weight of the total harvest of a plot was determined with an integrated balance directly on the plot harvester. Dry biomass production was determined by assessing dry weight – fresh weight ratios of the harvested biomass. For this a biomass subsample was collected for each plot and the fresh and dry weight (dried at 60°C for 48 h) were determined. After the harvest of the aboveground biomass in the central strip of a plot, the remaining standing biomass in a plot was mowed 7 cm above the ground and removed.

Roots

Belowground biomass of four grasses (DGe, DGl, LPe and LPl) was harvested six times per year, at the end of each drought period and six to eight weeks after drought release, from the respective treatment and control plots using a manual soil auger with a diameter of 7 cm. For each plot samples of the upper 14 cm soil were taken from two different spots (one sample directly from a tussock and one from in between tussocks) and pooled as one sample per plot. All samples were washed using a sieve with a mesh size of 0.5 cm × 0.5 cm and weighed after drying (at 60°C for 72 h).

Determining drought impacts on productivity

In order to allow the comparison of grassland productivity in the different treatments across the two years we standardized the productivity that occurred in between two harvest periods (i.e. during five weeks) for growth related temperature effects and calculated temperature-weighted growth rates for each of the six grasses (DMYTsum, see Menzi *et al.* (1991)). For this purpose, we determined temperature sums of daily mean air temperature above a baseline temperature of 5°C (Tsum) for each growth period (i.e. 5 weeks prior to harvest). Dry matter yield (DMY) of a given harvest was then divided by the temperature sum of the corresponding time period to obtain temperature-weighted growth rates (henceforth referred to simple as growth rate):

$$\text{DMYTsum} = \text{DMY}(\text{g/m}^2)/\text{Tsum}(^\circ\text{C}).$$

To determine the absolute change of growth (ACG) of a drought treatment on aboveground growth rate we calculated the difference between temperature-weighted growth rates in a drought treatment (drt) and the corresponding control (ctr):

$$\text{ACG} = \text{DMYTsum(drt)} - \text{DMYTsum(ctr)}.$$

To determine the relative change of growth (RCG) due to drought, we calculated percentage change of temperature-weighted growth rates:

$$\text{RCG} = 100 \times (\text{DMYTsum(drt)} / \text{DMYTsum(ctr)} - 1).$$

Annual ANPP as an average of the different grasses was determined by adding up the dry matter yields of the six harvests of a growing season. These data were not temperature-corrected (DMY).

We further calculated the sensitivity (S) of annual ANPP to the different drought treatments to quantify the response relative to the amount of precipitation change, as suggested by previous studies (Huxman *et al.*, 2004; Knapp *et al.*, 2017; Wilcox *et al.*, 2017):

$$\text{S} = (\text{DMY(ctr)} - \text{DMY(drt)}) / (\text{PPT(ctr)} - \text{PPT(drt)})$$

with PPT being the amount of precipitation in the treatment (drt) and control (ctr).

Data analysis

Relative and absolute changes in DMYTsum due to drought, the season of drought, and the tested grasses were analyzed using linear mixed-effects regression (Pinheiro

& Bates, 2000). Temperature-weighted growth rate (DMYTsum) was regressed on the fixed variables season (factor of three levels: spring, summer, fall), drought (factor of two levels: control, drought treatment) and grass (factor of six levels: LPe, LPl, DGe, DGl, LM, PP), including all interactions. To account for repeated measurements of the control plots over time (as the control for every seasonal drought treatment was the same), plot was specified as a random factor, thereby accounting for potential correlation of DMYTsum over time. DMYTsum was natural log-transformed prior to analysis to improve homogeneity and normal distribution of residual variance. This transformation also implies that the regressions provide the inference to relative changes in DMYTsum, namely RCG. A temporal compound symmetry correlation structure was initially imposed on the residuals, yet, it turned out that the estimated correlation parameter was very small. A likelihood ratio test indicated its non-significance ($p>0.5$) and it was finally omitted. However, inspection of residuals revealed clear differences in their variance among seasons and control and drought plots, and the residual variance parameter was defined as $\mathrm{Var}(e_{jk}) = \sigma^2\delta_{jk}^{\ 2}$, with δ being a ratio to represent $j \times k$ variances, one for each of three seasons j under control and drought conditions k (Pinheiro & Bates, 2000). The R^2 of explained variance of fixed effects was computed following (Nakagawa & Schielzeth, 2013). This model was applied to DMYTsum at each second growth period under drought and the second post-drought growth period in 2014 and 2015.

Root dry weight was analyzed in a similar way, i.e. it was natural log-transformed prior to analysis and the same explanatory factors were applied except that the factor grass had only four levels (only LPe, LPl, DGe and DGl measured). Here, estimation of a single residual variance parameter e_i was sufficient to fulfill the model assumptions.

Annual ANPP was analyzed by one-way analysis of variance. The first factor season-treatment consisted of the four levels control, spring drought, summer drought, and fall drought. The second factor grass consisted of six levels, representing the six grasses.

All statistical analyses were done using the statistical software R, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria, 2018). Graphics were implemented with the package *ggplot2*, version 2.1.0 (Wickham, 2016).

Results

Precipitation, evapotranspiration and soil water potential

Over the entire growing season the year 2015 was exceptionally dry, while 2014 showed normal climatic conditions for the experimental site. The difference between rainfall (634 and 568 mm for 2014 and 2015, respectively) and evapotranspiration (356 and 447 mm for 2014 and 2015, respectively) was 278 mm in 2014 and only 121 mm in 2015 for the unsheltered control plots. The shelter periods reduced the total annual precipitation in the different treatments between 17.9 % and 37.0 % and the precipitation of the growing season (duration of the experiment, approx. March – November) by between 23.1 % and 45.8 % (see Table 1).

Table 1: Amount of rainfall fallen in the experiment and associated amount of excluded rainfall during the sheltered drought periods in the years 2014 and 2015. Growing season precipitation refers to the period of time between the set-up of the shelters and the last harvest of each year.

2014				
annual precipitation	growing season precipitation	spring	summer	fall
		excluded precipitation (mm)		
937.1	717.9	167.4	308.8	241.7
		excluded precipitation annually (%)		
		17.9	33.0	25.8
		excluded precipitation in growing season (%)		
		23.2	43.0	33.7
2015				
annual precipitation	growing season precipitation	spring	summer	fall
		excluded precipitation (mm)		
801.9	648.5	296.9	204.7	149.9
		excluded precipitation annually (%)		
		37.0	25.5	18.7
		excluded precipitation in growing season (%)		
		45.8	31.6	23.1

In 2014, Ψ_{Soil} was severely reduced in the drought treatments and reached values around the permanent wilting point (-1.5 MPa) for the entire second half of the sheltered periods in all treatments (spring, summer, fall) (Fig. 2b-e, Table 2). Due to low rainfall in June 2014 Ψ_{Soil} dropped not only in the sheltered summer drought treatment, but also in the control and the fall drought treatment (that was not yet sheltered). Ψ_{Soil} recovered in the treatment plots after each sheltered period and reached Ψ_{Soil} values comparable to the ones in the control plots. Because of the lack of rain in June 2014 the full rewetting of the spring drought treatment occurred only in the second post-drought growth period after the spring drought shelter period, while after the summer drought treatment rewetting occurred already in the first post-drought growth period.

In 2015, drought treatments reduced Ψ_{Soil} in all seasons (Fig. 2g-k). However, an intense rain event caused some surface runoff in the field on May 1st 2015, which partly interrupted the spring drought treatment. Still, for the second growth period of the spring drought treatment of 2015 the median of Ψ_{Soil} was at -0.77 MPa, a value comparable to that of the second growth period of the summer drought treatment (-0.83 MPa) (Table 2). In 2015, Ψ_{Soil} reached lower values during the shelter period in the fall treatment than during the shelter period in the spring and summer treatments. Due to a lack of rain in 2015 Ψ_{Soil} recovered only partly after the end of the shelter period in the spring and summer drought treatments and remained significantly below that of the control plots for both post-drought growth periods (Table 2). Watering of the control plots during natural dry conditions lead to quick increases in Ψ_{Soil} to values close to saturation (=0 MPa). Daily mean air temperature under the rainout shelters was between 2.9°C lower and 3.5°C higher than in the control.

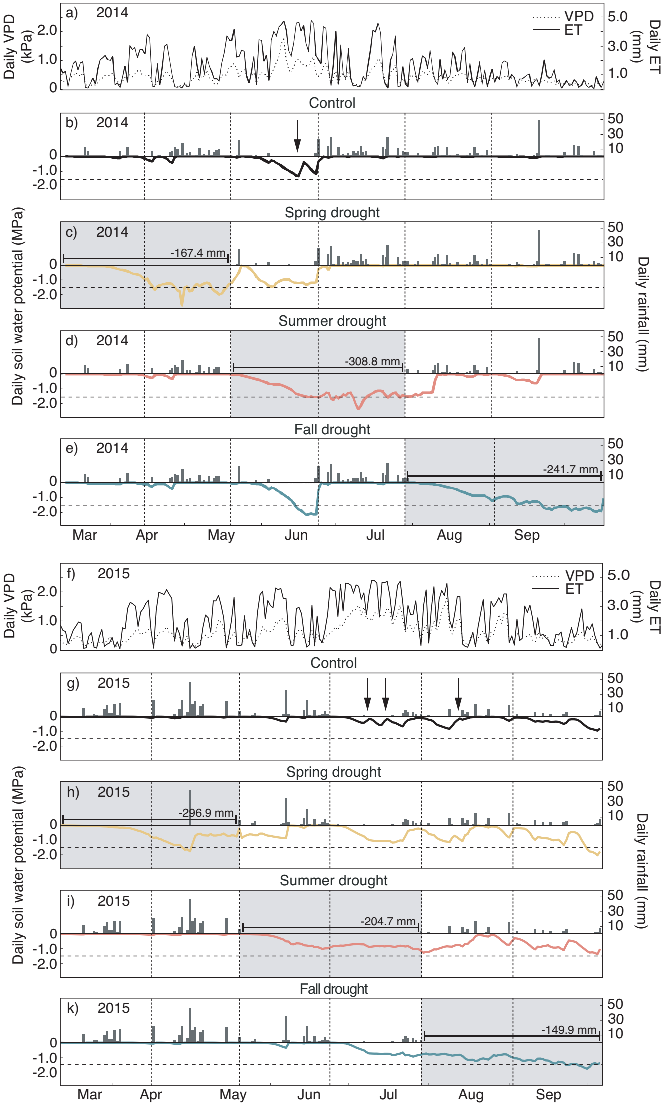

Fig. 2: (a, f) Daily evapotranspiration (ET) and vapor pressure deficit (VPD), (b-e, g-k) daily rainfall and soil water potential in 10 cm depth (Ψ_{Soil}) over the growing seasons 2014 (a-e) and 2015 (f-k) for the control and drought treatments (sensors per treatment: n=8). Grey shaded areas represent the experimental drought when rainfall was excluded (amount of excluded rainfall denoted). Dashed horizontal line shows permanent wilting point (Ψ_{Soil}=-1.5MPa). Dashed vertical lines represent dates of harvest. Arrows indicate watering events (in control plots only).

Table 2: (a) Median of soil water potential (Ψ_{Soil}) and (b) average air temperature during the two growth periods of the drought treatments and the two post-drought growth periods as well as the corresponding periods of the rain-fed control. n.a.: not available.

a)

Growth period	Control			Treatment		
	spring	summer	fall	spring	summer	fall
2014	MPa					
1st drought	-0.03	-0.41	-0.01	-0.09	-0.72	-0.73
2nd drought	-0.01	-0.01	-0.01	-1.44	-1.44	-1.61
1st post-drought	-0.41	-0.01	-0.01	-1.1	-0.05	-0.01
2nd post-drought	-0.01	-0.01	n.a.	-0.01	-0.02	n.a.
2015	MPa					
1st drought	-0.01	-0.02	-0.14	-0.08	-0.45	-0.85
2nd drought	-0.01	-0.25	-0.34	-0.77	-0.83	-1.34
1st post-drought	-0.02	-0.14	n.a.	-0.57	-0.73	n.a.
2nd post-drought	-0.25	-0.34	n.a.	-0.7	-0.88	n.a.

b)

Growth period	Control			Treatment		
	spring	summer	fall	spring	summer	fall
2014	°C					
1st drought	10.3	18.0	16.6	11.0	19.0	17.3
2nd drought	10.9	18.0	15.2	11.5	18.7	15.8
1st post-drought	18.0	16.6	7.1	18.0	16.6	7.1
2nd post-drought	18.0	15.2	n.a.	18.0	15.2	n.a.
2015	°C					
1st drought	7.1	16.2	20.3	7.6	16.9	20.5
2nd drought	13.3	22.7	13.0	14.4	23.7	13.5
1st post-drought	16.2	20.3	n.a.	16.2	20.3	n.a.
2nd post-drought	22.7	13.0	n.a.	22.7	13	n.a.

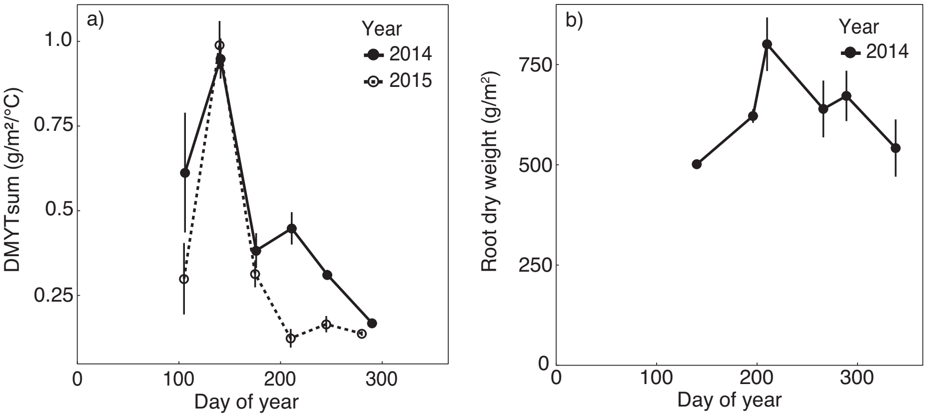

Fig. 3: (a) Temperature-weighted growth rates (DMYTsum) of aboveground biomass of rain-fed control plots in 2014 and 2015; values are means across all six investigated grasses and four replicates (n=6, ± se) and b) belowground biomass of rain-fed control plots in 2014; values are means across the four grasses *L. perenne* early (LPe) and late (LPl) flowering and *D. glomerata* early (DGe) and late (DGl) flowering (n=4, ± se).

Table 3: Summary of analysis for the effects of season, drought treatment, grass, and their interactions on temperature-weighted growth rates (DMYTsum, natural log-transformed) from the second growth period during drought (weeks six to ten). The inference (*F*- and *p*-values) and the R^2 refer to the fixed effects from the linear mixed model. df_{num}: degrees of freedom term, df_{den}: degrees of freedom of error.

			2014		2015	
Effect	df_{num}	df_{den}	*F*-value	*p*	*F*-value	*p*
Season (spring, summer, fall)	2	36	1051.1	<0.001	2655.3	<0.001
Treatment (control vs. drought)	1	72	341.9	<0.001	642.9	<0.001
Grass	5	72	9.4	<0.001	14.2	<0.001
Season × Treatment	2	72	25.9	<0.001	366.2	<0.001
Season × Grass	10	36	6.8	<0.001	10.3	<0.001
Treatment × Grass	5	72	2.9	0.018	2.0	0.094
Season × Treatment × Grass	10	72	3.3	0.001	3.4	0.001
R^2			0.901		0.965	

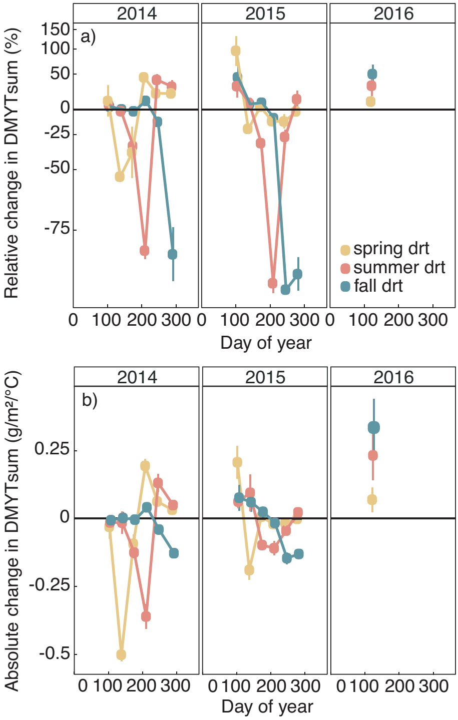

Fig. 4: (a) Relative (RCG) and (b) absolute (ACG) changes in temperature-weighted growth rates (DMYTsum) of the respective drought (drt) treatment compared to the control (ctr) for 2014, 2015 and 2016. Values are means ± se across all six investigated grasses each in four replicates. Values below the horizontal black line indicate reduced growth compared to the control. Values above the line indicate an increase of growth. RCG=100×(DMYTsum(drt)/DMYTsum(ctr))-1); displayed on log-scale); ACG=DMYTsum(drt)−DMYTsum(ctr).

Varying growth rates throughout the growing season

The temperature-weighted growth rates of the investigated six grasses in the control plots showed a clear seasonal pattern (Fig. 3a). In both years, it was highest during the second growth period in spring and sharply declined to values that were two- to eight-fold smaller in summer and fall. Except for the second growth period growth rates of the grasses were lower in 2015 than in 2014. Root biomass increased towards summer and slightly decreased after summer in 2014 (Fig. 3b).

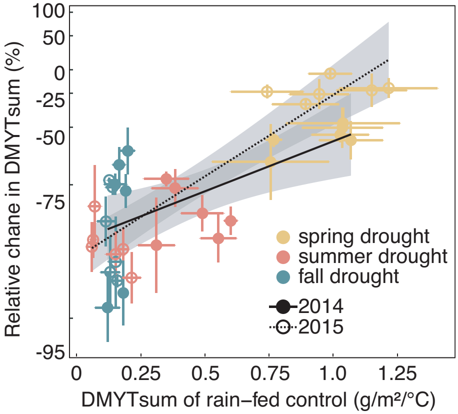

Fig. 5: Relative changes of temperature-weighted growth rate due to drought (RCG; %) as a function of temperature-weighted growth rate (DMYTsum) of the corresponding rain-fed control plots (g/m^2/°C). Values are means ± se for all six investigated grasses each in four replicates.

Seasonality of drought resistance

The growth rates of the six grasses were barely affected by the exclusion of rain during the first five weeks of sheltering (Fig. 4). However, during the second sheltered growth period (weeks six to ten) the drought treatments strongly reduced temperature-weighted growth rates in all seasons, in both years, and in relative and absolute terms (Figs. 4, 5 and 6, Table 3). In both years, the relative drought-induced changes in growth rates compared to the controls were smallest in spring (2014: -51%, 2015: -20%) and clearly larger in summer (2014: -81%, 2015: -85%) and fall (2014: -77%, 2015: - 84%) (Fig. 4a, Table 3; season x treatment $p<0.001$). As such, the drought resistance of the grasses throughout the growing season was largest in spring and positively correlated with their productivity (Fig. 5). This pattern was generally observed for all six grasses tested (Fig. 6a) even though there was a significant season × treatment × grass interaction (Table 3). In 2014, this interaction mainly derived from DGl and PP showing an exceptionally large drought induced growth reduction in fall. In 2015, it was explained by an especi-

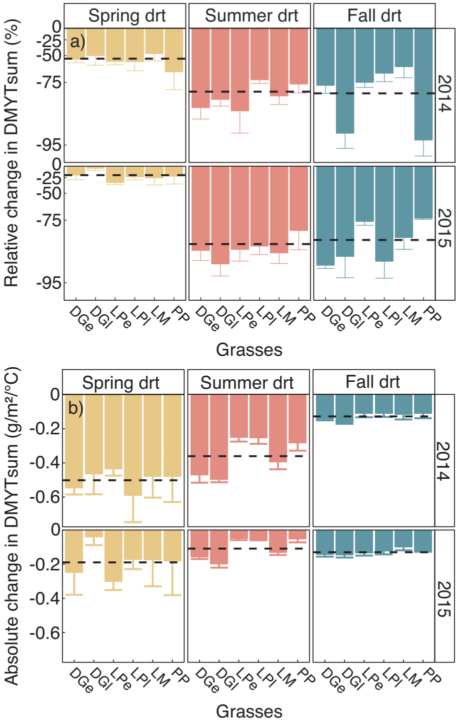

Fig. 6: (a) Relative (RCG) and (b) absolute (ACG) changes in temperature-weighted growth rates (DMYTsum) for the second growth period (weeks six to ten) of the respective drought (drt) treatment for 2014 and 2015 for the individual grasses. Values are means of four replicates ± se. Dashed black lines represent the means across all grasses. See Fig. 4 for additional explanation.

ally low drought response of DGl in spring and strong responses of DGl in summer and LPe and PP in fall (Fig. 6a).

In 2014, the absolute drought-induced reduction of growth across all six grasses was largest in spring (-0.5 g/m^2/°C), followed by summer (-0.4 g/m^2/°C) and was lowest in the fall (-0.1 g/m^2/°C) (Fig. 4b). Likewise, in 2015 the absolute reduction of the growth rate in the drought treated plots was largest across the six grasses in spring (-0.2 g/m^2/°C), but slightly lower in summer (-0.1 g/m^2/°C) and fall (-0.1 g/m^2/°C).

The average standing root biomass across four of the grasses was not significantly affected by any of the drought treatments of 2014 (Fig. 7).

Table 4: Summary of analysis for the effects of season, drought treatment, grass, and their interactions on temperature-weighted growth rates (DMYTsum, natural log-transformed) from the second post-drought growth period (weeks six to ten). See Table 3 for additional explanation.

			2014		2015	
Effect	df_{num}	df_{den}	*F*-value	*p*	*F*-value	*p*
Season (spring, summer, fall)	2	36	783.4	<0.001	1428.6	<0.001
Treatment (control vs. drought)	1	72	63.5	<0.001	25.5	<0.001
Grass	5	72	18.4	<0.001	39.4	<0.001
Season × Treatment	2	72	1.8	0.180	16.6	<0.001
Season × Grass	10	36	15.7	<0.001	9.6	<0.001
Treatment × Grass	5	72	0.9	0.517	6.4	<0.001
Season × Treatment × Grass	10	72	2.2	0.025	0.8	0.621
R^2			0.810		0.944	

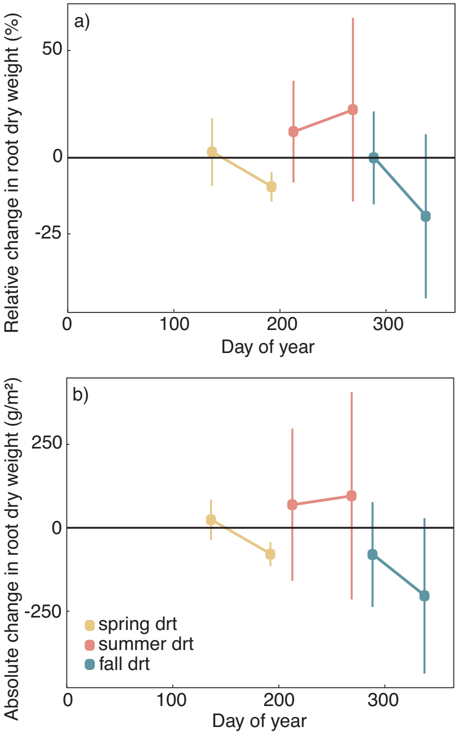

Fig. 7: (a) Relative and (b) absolute changes in root dry matter at the end of each drought treatment and after six to eight weeks after drought-release in 2014. Values are means ± se of four grasses of *L. perenne* (LPe and LPl) and *D. glomerata* (DGe and DGl) each in four replicates.

Seasonality of post-drought resilience

When compared to corresponding controls, relative (and absolute) changes in temperature-weighted growth rates after drought release showed positive treatment effects in 2014 (Fig. 8, Table 4). Across all six grasses, the relative increases in post-drought growth rates were 41% after the spring drought treatment, 31% after the summer drought treatment, and 53% after the fall drought treatment, and did not differ among the seasons (Table 4; season × treatment *p*=n.s.). In 2015, the relative increases in post-

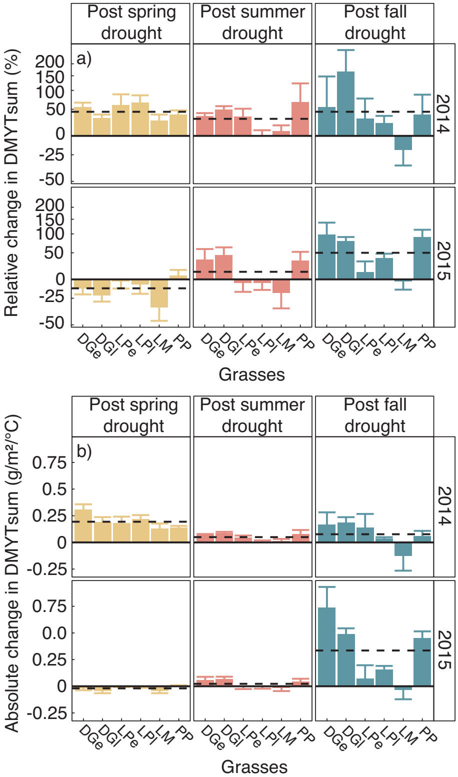

Fig. 8: (a) Relative (RCG) and (b) absolute (ACG) changes in temperature-weighted growth rates (DMYTsum) for the second post-drought growth period (weeks six to ten) in 2014 and 2015 after the respective drought (drt) treatment for the individual grasses. Values are means of four replicates ± se. Post-drought growth period of the fall drought treatment is the first growth period of the following year. Dashed black lines represent the means across all grasses. See Fig. 4 for additional explanation.

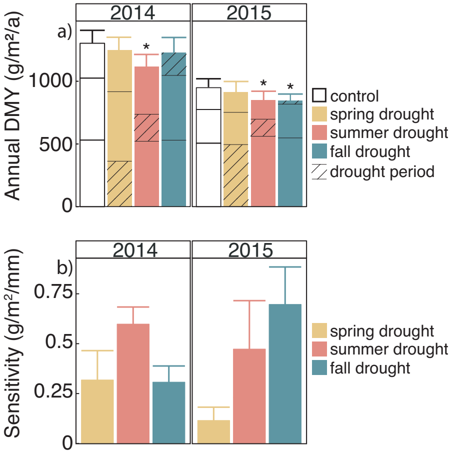

Fig. 9: (a) Annual dry matter yield under rain-fed control and under the three seasonal drought treatments and (b) sensitivity of the three seasonal drought treatments in the years 2014 and 2015. Values are means ± se across all six investigated grasses each in four replicates. Bars in (a) are stacked according to growth in spring (bottom part), summer and fall (top part). Significant differences to the control are marked with * ($p<0.05$).

drought growth rates were 5% after the spring drought treatment, 15% after the summer drought treatment, and 52% after the fall drought treatment, and did differ among the seasons (Table 4; season × treatment $p<0.001$). Increased growth rates were also observed in the first harvest in 2015 and 2016 for all the plots that had received a drought treatment in 2014 and 2015, respectively (Fig. 4). In this first harvest of 2015, growth rate increases were 110% after the spring, 36% after the summer and 53% after the fall drought treatments of 2014. In the first harvest of 2016, growth rate increases were 10% after the spring, 31% after the summer and 51% after the fall drought treatments of 2015.

When compared across the different grasses, the only grass that tended to have a weaker resilience (lower or no increase of growth rate during post-drought) was LM (Fig. 8); but there was no significant difference among the grasses (Table 4; treatment x grass *p*=n.s.). In 2015, again LM showed the weakest resilience of all the grasses after all drought treatments, the effect being significant (Table 4; treatment x grass p<0.001).

Root dry weight of the treatment plants showed no alterations in growth compared to the control in either of the post-drought periods (Fig. 7).

Effects of seasonal drought on annual biomass production

The cumulative annual aboveground biomass production (i.e. annual ANPP) of the controls averaged across all six grasses differed strongly between the two years (Fig. 9a), with 2014 (1303 g/m^2/a) being 37% more productive than 2015 (949 g/m^2/a). The strong reduction in biomass production in 2015 was most probably related to the naturally occurring lack of rain in summer and fall (Fig 2). This is evident from the two spring growth periods being equally productive in the unsheltered plots (control, summer and fall drought) in 2015 and in 2014 (Fig. 9a). The annual ANPP of the treatments was significantly different from the control in both years. In 2014, the largest drought effect on the annual ANPP across all grasses resulted from the summer treatment, which reduced productivity significantly by 14% (185 g/m^2) compared to the control. Spring and fall drought treatments in 2014 resulted in a non-significant 4% (53 g/m^2) and 6% (74 g/m^2) reduction of annual ANPP across all grasses, respectively. In 2015, drought treatments in the summer and fall significantly caused a 10% and 11% reduction of annual ANPP across all grasses (-97 g/m^2 and -105 g/m^2), respectively, while the spring drought treatment reduced annual ANPP across all grasses by only 4% (34 g/m^2), which was not significant (Fig. 9a).

The sensitivity of annual ANPP to drought differed between the treatments in both years of the experiment. In 2014, the annual ANPP was most sensitive to drought in the summer, while annual ANPP was less sensitive to rainfall reduction in the spring, but also fall (Fig. 9b). In 2015, the sensitivity increased within the growing season so that annual ANPP was least sensitive to spring drought and most sensitive to fall drought (Fig. 9b).

Discussion

In our study we experimentally assessed if the drought resistance and resilience of six different temperate perennial C3 grasses varies throughout the growing season and if the timing of a drought event, thus, has an influence on drought induced reductions in annual NPP of these grasses. All six temperate grasses showed a clear seasonal pattern of drought resistance in both years. The drought-induced reduction of growth was smaller under spring drought (-20% and -51% for the two years) than under summer and fall droughts (between -77% and -87%). Thus, the investigated grasslands were more resistant to drought in the spring when productivity of temperate grasses is generally the highest and they were least resistant in summer and fall, when their productivity is much lower. Moreover, the examined grasslands did not show any negative legacy effects such as a prolonged suppression of growth after rewetting following the end of the drought treatments. In contrast, after the release of drought temperature-weighted growth rates of the grasses in the treatment plots surprisingly outperformed the growth rates of the grasses in the controls for extended periods of time. This suggests a high resilience of all six grasses that we investigated. As a consequence of the high resilience, the seasonal drought treatments resulted in only moderate drought-induced reductions in annual aboveground NPP between -4% to -14% – despite the strong immediate effects of drought – and no clear effects of the timing of drought on annual NPP were detected. With this our study shows (i) that the resistance of growth rates in different grasses to drought varies throughout the growing season and is positively correlated with growth rates in the control, (ii) that positive legacy effects of drought on plant productivity indicate a high resilience of temperate C3 grasses throughout the entire growing season, and (iii) that the high resilience can strongly compensate for immediate seasonal drought effects on productivity, resulting in total annual NPP that is only marginally reduced in the drought treated plots compared to the controls.

Differences in the climatic conditions between the two years

While the first experimental year (2014) was characterized by more or less normal climatic, and thus, growth conditions, the summer of 2015 was exceptionally dry in all of central Europe (Orth *et al.*, 2016; Dietrich *et al.*, 2018). These conditions led to a reduction of the annual NPP of the control plots by 37% in 2015 compared to 2014 (Fig. 9a). The lack of rain in the second half of the 2015 growing season, i.e. between the third harvest in June and the last harvest in October (Fig. 2) was of importance for our experiment, especially for the response of the treatments during the recovery phase after the removal of the shelters. In this period, the amount of rainfall was only 153 mm in 2015 while it was 405 mm in 2014. Thus, positive legacy effects directly following drought treatments were much smaller or absent following the spring and summer treatments in 2015 due to a missing rewetting (Figs. 2, 4 and 8). Yet, strong positive legacy effects in response to the 2015 treatments were observed in the first harvest of 2016 when the experimental site was fully rehydrated. This highlights the general occurrence of positive drought legacy effects in the investigated grasslands once the soil moisture has recovered from the drought treatments.

Intense rains between the first and second harvest of the year 2015 caused some water flow into the treatments. This resulted in a partial reduction of drought stress in the treatment plots (Fig. 2h). Yet, the median of the soil water potential was still clearly reduced in the treatment plots compared to the control and, consequently, we observed a reduction of growth rates in the second spring harvest in 2015 despite this event (Figs. 4, 6). We therefore conclude that the partial reduction in drought stress did weaken the immediate drought response during the growth period concerned, but that this does not question the overall drought responses of the grasslands that we report here.

Grasses were most resistant to drought in spring, the most productive part of the growing season

Previous studies have indicated that the timing of drought is relevant for the reduction of annual NPP of ecosystems (Bates *et al.*, 2006; Nippert *et al.*, 2006; La Pierre *et al.*, 2011; Denton *et al.*, 2017). It has been argued that the variable drought sensitivity of ecosystems throughout the growing season could be linked to different phenological stages of dominant plant species, where plants in reproductive stages and periods of high growth are particularly susceptible to drought (O'Toole, 1982; Bates *et al.*, 2006; Heitschmidt & Vermeire, 2006; Craine *et al.*, 2012; Dietrich & Smith, 2016). We found, however, that relative reductions in temperature-weighted growth rates were lowest in the spring treatments 2014 and 2015 as compared to the summer and fall treatments. The highest resistance of plant growth rates to drought occurred, thus, when the plants showed the highest growth rates in the control and when the investigated grasses were in their reproductive stages (Fig. 5). With this, our findings are in contrast to previous studies that have suggested temperate grasslands and crops to be particularly susceptible to drought early in the growing season when their growth rates are the highest and plants are in reproductive stages (O'Toole, 1982; Bates *et al.*, 2006; Heitschmidt & Vermeire, 2006; Robertson *et al.*, 2009; Jongen *et al.*, 2011; Craine *et al.*, 2012; Dietrich & Smith, 2016). Our study does support, however, findings of Simane *et al.* (1993) and El Hafid *et al.* (1998), who detected that spring droughts have the least impact on annual productivity of wheat. Importantly, most of the previous studies that have reported the effects of drought timing on grasslands or other ecosystems report effects on annual NPP but have not differentiated immediate and long-term legacy effects of drought events as we did in our study. As drought impacts on annual NPP combine immediate and post -drought legacy effects, it is difficult to directly compare the results we present here on variably seasonal drought resistance of temperate C3 grasses to previous work reporting the influence of drought timing on annual NPP.

One possibility for the higher drought resistance of grasses during spring is that grasses invest more resources towards the stress resistance of their tissue in this part of the growing season when they have not only the largest growth rates, but also reproduce. Such a resource allocation strategy could allow drought stressed grasses to remain physiologically active in this critical part of the growing season. Osmotic adjustment is one mechanism that reduces the effects of drought on the physiological performance of the plant (Sanders & Arndt, 2012). This is achieved through the active accumulation of organic and inorganic solutes within the plant cell. Thus, osmotic potential increases and the plant can withstand more negative water potentials in the cell while maintaining its hydraulic integrity (Sánchez *et al.*, 1998). Santamaria *et al.* (1990) found that early- and late flowering cultivars of *Sorghum bicolor* L. developed a different pattern of osmotic adjustment (continuous increase of osmotic adjustment vs. first increase and later decrease of osmotic adjustment), hinting that drought tolerance may vary between seasons. In a companion paper we report physiological data for the six grasses from the same experiment. We show that at a given soil water potential, foliar water potentials were less negative and stomatal conductance was higher in plants drought stressed in the spring compared to plants drought stressed in the summer or fall (Hahn *et al.*, in prep). This suggests, indeed, that for a given drought level, grasses remain physiologically more active in the spring than in the summer or fall. The exact physiological mechanisms that explain the higher drought resistance of the investigated grasslands in the spring and their higher drought susceptibility in the summer and fall remain, yet, unknown and require further detailed ecophysiological and biochemical assessments.

An alternative explanation for different immediate drought effects on growth rates throughout the growing season are experimental artefacts causing different experimentally induced drought severities throughout a growing season. This could be by either residual moisture dampening the experimentally induced drought more in the spring than in the summer or fall. Alternatively, higher evaporative demand of the atmosphere in the summer compared to the spring or fall could have enhanced experimentally induced drought effects in the summer. De Boeck *et al.* (2011) explain for example the higher drought susceptibility of growth in three herbs in the summer compared to spring by a higher evaporative demand of the atmosphere in the summer compared to spring or fall. In our study, however, soil water potential data indicate that ten weeks of drought treatment reduced plant available water in the soil to mostly equal levels in spring, summer and fall (Fig. 2). In addition, we found only small differences in median VPD between the spring, summer and fall drought treatment period (Fig. 2). This suggests that stronger drought stress in summer and fall compared to spring cannot explain alone the different resistances of plant growth to drought throughout the growing season. Along these lines, Denton *et al.* (2017), who performed a similar experiment as we report here but in a C4 grassland in North America, also did not find that these seasonal differences in the experimentally induced drought severity are the reason for variable drought effects on the growth rates throughout the growing season.

No increased root biomass in the top soil layer

In the entire experiment, root biomass increased only in one out of the four investigated grasses (DGe) in response to drought in summer as well as in the post-summer drought period. This confirms the findings of Gill *et al.* (2002), Byrne *et al.* (2013) and Denton *et al.* (2017), who did not find any changes in belowground biomass in response to drought. In a similar setting, Gilgen & Buchmann (2009) found no changes in belowground biomass to simulated summer drought in three different temperate grassland sites (from lowland to alpine grassland). While Denton *et al.* (2017) ascribe the missing drought response in belowground biomass to modest precipitation alterations in their experiment, we can exclude this factor in our experiment since the soil water potential under drought was significantly reduced compared to the soil water potential in the controls in every season. Contrary to that, several studies have shown that drought can maintain or increase root growth while inhibiting shoot growth (Saab *et al.*, 1990; Davies & Zhang, 1991; Hofer *et al.*, 2017a). In an experiment by Jupp & Newman (1987), *L. perenne* increased lateral root growth under low Ψ_{Soil} indicating an increased investment in root growth under water limited conditions. In our experiment, the *L. perenne* grasses did not show a trend towards increased investment in root growth, neither during drought nor after drought-release, contradicting the results of Jupp & Newman (1987). Such differences in the response of root biomass in different studies as described above may derive from the soil layer that was investigated. Hofer *et al.* (2017a) have shown that the response of root growth into ingrowth bags depended on the soil depth: root growth of *L. perenne* decreased in the top soil layer (0-10 cm), but increased in deeper soil layers of 10-30 cm. Thus, the superficial root sampling (0-14 cm) in our experiment might mask increased root growth in deeper soil.

Positive legacy effects of drought periods

Several previous studies have suggested that drought events can lead to negative legacy effects on the productivity of ecosystems (Sala *et al.*, 2012; Reichmann *et al.*, 2013; Petrie *et al.*, 2018). We found, however, that growth rates of previously drought-stressed plots were significantly larger than in the corresponding control plots after rewetting, indicating positive legacy effects and a high resilience of the investigated grasses (Figs. 4 and 8). Interestingly, we did not only observe growth rates that were larger in the treatment plots than in the control plots immediately after the drought release, but observed larger growth rates in all treatment plots compared to the control plots even in the first harvests of the following growing season (Fig. 4). This pattern was consistent for both years of the experiment. Bloor & Bardgett (2012) and also Denton *et al.* (2017) found that drought events promote soil fertility and nutrient retention following drought release.

Likewise, Gordon *et al.* (2008) found an increase in microbial activity after a rewetting event, possibly leading to a rapid and sudden influx of plant available nitrogen in the soil (Schimel & Bennett, 2004; Mackie *et al.*, 2018). Hofer *et al.* (2017a) also attributed growth increases relative to control plots in post-drought periods to nitrogen availability in the soil and Karlowsky *et al.* (2018) found evidence that interactions between plants and microbes increase plant nitrogen uptake in grasslands after rewetting events. It could, thus, be that the enhanced productivity in the treatment plots following drought release is the result of increased microbial activity leading to enhanced nitrogen availability and/or changes in resource limitation following drought release as suggested by Seastedt & Knapp (1993) in their Transient Maxima Hypothesis.

We applied nitrogen fertilizer in our experiment to each plot after each harvest, also at the beginning and in the middle of a drought treatment. Since we applied the fertilizer in form of water soluble pellets, it is possible that nitrogen fertilizer pellets accumulated in the drought-treated plots during the treatment phase. The rewetting of the soil could have resulted in a massive release of nitrogen fertilizer from these pellets so that plant growth rates in formerly drought-stressed plots were stimulated by the release of this fertilizer and, thus, larger than those of the control plots. However, Hofer *et al.* (2017a) observed strongly increased N availability and plant growth rates after drought release not only in plots that received mineral fertilizer during the drought treatment period, but also in plots that did not receive any N fertilizer during drought. We suggest, therefore, that the release of accumulated fertilizer nitrogen in the treatment plots might explain some, but not all post-treatment growth responses in the formerly drought treated plots in our study.

Hagedorn *et al.* (2016) have shown that rewetting events trigger intrinsic processes that lead to a sudden increase of photosynthesis in young beech trees. Moreover, Arend *et al.* (2016) found a rapid stimulation of photosynthesis immediately after rewetting that continued until the end of the growing season, partly compensating the loss of photosynthetic activity during drought. Hofer *et al.* (2017b) found an increased root mass and increased water soluble carbohydrate reserves in the stubbles of drought stressed *L. perenne* at the end of a drought stress period. Both of which could have contributed to increased growth rates observed in their study once rewetting had occurred. Also, drought-induced shifts in plant phenology could lead to a shift in high productive stages, e.g. leading to peak growth rates not in spring, but in summer (O'Toole & Cruz, 1980). With the data we collected throughout our experiment we cannot clearly identify the mechanisms behind the strong post-drought growth increase that extended even into the next growing season. In the end, several biogeochemical and ecophysiological mechanisms might be responsible for the overcompensation of growth following drought release.

The grasses only slightly differed in drought resistance and resilience

During the seasonal drought events the six tested grasses showed a mostly universal response with only slight and not consistent differences in their growth rate reductions.

Post-drought legacy effects differed among the different grasses in the second year (grass x treatment; p=n.s. for 2014 and p<0.001 for 2015). *D. glomerata* and *P. pratensis* showed a high potential for resilience and overcompensation after drought, while *L. multiflorum* generally showed the lowest resilience. Wang *et al.* (2007) found that plant communities consisting of less productive species were more resistant to drought than plant communities consisting of more productive species. The fact that interspecific differences in the responses to the drought stress and to the following rewetted post-drought period in our study were smaller than in other studies, may be related to the fact that all six tested grasses belong to a relatively narrow functional group of productive fast growing grasses with high demands for mineral N in the soil. The availability of mineral N in the soil was found to be a key factor for the response during as well as after drought for non-leguminous species (Hofer *et al.*, 2017a,b)

Small to moderate impact of seasonal drought on annual aboveground net primary production

Although the immediate effects of drought on growth rates were severe in all three seasons in our study, the overall effects on total annual ANPP of 4 to 14% were only small to moderate compared to drought effects observed in other studies (Wu *et al.*, 2011; Wilcox *et al.*, 2017; Gherardi & Sala, 2019) (Fig. 9a). We also did not find any consistent effects of the drought timing on annual NPP, contrary to other studies (Nippert *et al.*, 2006; La Pierre *et al.*, 2011; Denton *et al.*, 2017; Petrie *et al.*, 2018). This is likely a consequence of the small overall drought effects on annual ANPP in our study.

The small drought effects on annual ANPP that we report here can be explained by the high resilience of growth rates in the treatment plots following the drought release. This is in particular evident in the spring treatment, where we observed on the one side the largest absolute reduction in growth in response to drought, but at the same time also the strongest positive legacy effects after drought, leading to relatively small total drought effects on annual aboveground NPP. Because the fall drought treatment period lasted until the end of the vegetation period the positive post-drought legacy effects for this treatment were not included in the calculation of annual biomass production. Nevertheless, the fall drought treatment in 2014 did also not strongly affect the annual aboveground NPP. This is because the growth period affected by the fall drought treatment, was the least productive part of the growing season, and, thus contributed only little to the annual productivity.

The overall effect of drought on annual ANPP might also be small compared to other studies, because our study was conducted in highly productive grasslands that, according to best practice management, were harvested six times in the growing season. The drought treatments occurred, however, only in two out of these six growth periods throughout the growing season. In addition, the first sheltered growth period generally did not show a reduced growth rate (Fig. 4), because the soil with its water holding capacity acted as a buffer. With the absence of negative legacy effects, the impact of the immediate drought effect of one drought stressed growth period on annual NPP was therefore diluted by the five other harvests of the vegetation period (Finn *et al.*, 2018).

The majority of studies that have assessed the impact of drought on grassland productivity have either assessed immediate drought effects, i.e. drought resistance (Kahmen *et al.*, 2005; Wang *et al.*, 2007; Walter *et al.*, 2012; Bollig & Feller, 2014), or have assessed the net effects of drought on annual NPP (Wu *et al.*, 2011; Wilcox *et al.*, 2017; Gherardi & Sala, 2019). Our study highlights that it is important to also quantify immediate and post-drought effects – even in the following growing season – if the causes of drought reduced annual productivity are to be understood.

Effects of drought on annual aboveground NPP of grasslands have been shown to vary, depending on the severity of the experienced drought (Vicca *et al.*, 2012; Wilcox *et al.*, 2017), the ecosystem type (Byrne *et al.*, 2013; Sala *et al.*, 2015; Wilcox *et al.*, 2017; Gherardi & Sala, 2019), the intensity of land use (Walter *et al.*, 2012; Vogel *et al.*, 2012), the plant functional composition (Gherardi & Sala, 2015; Hofer *et al.*, 2016, 2017a; Mackie *et al.*, 2018), or the biodiversity of an ecosystem (Kahmen *et al.*, 2005; Isbell *et al.*, 2015; Wagg *et al.*, 2017). In accordance with work in C4 grasslands, our study shows that the timing of a drought event in the growing season is crucial for the immediate effects of a drought on grassland productivity. Importantly, however, our study also shows that strong positive legacy effects can occur after rewetting and that these legacy effects are even important in spring of the next year. These effects can partially compensate the strong immediate drought effects and lead relatively small overall seasonal drought effects on annual ANPP.

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Supplementary

Table S1: Summary of analysis for the effects of season, drought treatment, grass, and their interactions on root biomass (natural log-transformed) during drought and the post-drought period in 2014. See Table 3 for additional explanation.

			during drought		post-drought	
Effect	df_{num}	df_{den}	*F*-value	*p*	*F*-value	*p*
Season (spring, summer, fall)	2	24	14.4	<0.001	20.8	<0.001
Treatment (control vs. drought)	1	48	0.3	0.572	0.4	0.553
Grass	3	48	6.5	<0.001	8.5	<0.001
Season × Treatment	2	48	0.2	0.825	3.8	0.030
Season × Grass	6	24	3.9	0.007	5.2	0.002
Treatment × Grass	3	48	2.1	0.113	5.2	0.003
Season × Treatment × Grass	6	48	1.9	0.104	4.8	<0.001
R^2			0.486		0.619	

Table S2: Summary of analysis of variance for the effects of season-treatment[1], grass, and their interaction on annual dry matter yield in 2014 and 2015.

		2014		2015	
Effect	df	*F*-value	*p*	*F*-value	*p*
Season-treatment	3	9.4	<0.001	4.9	0.007
Grass	5	64.3	<0.001	29.2	<0.001
Season-treatment × Grass	15	0.8	0.687	1.4	0.190
R^2		0.781		0.619	

[1] With the four levels (i) control, (ii) spring drought, (iii), summer drought, and (iv) fall drought.

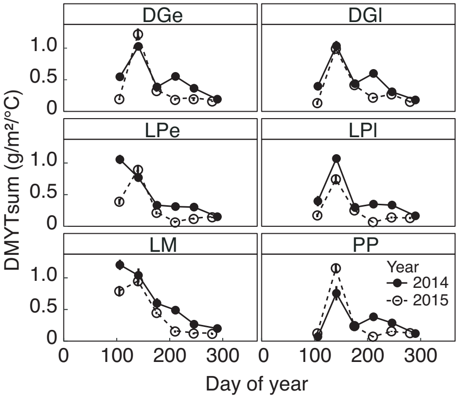

Fig. S1: Temperature-weighted growth rates (DMYTsum) of aboveground biomass of rain-fed control plots for the individual grasses in 2014 and 2015; values are means across four replicates ± se (n=4).

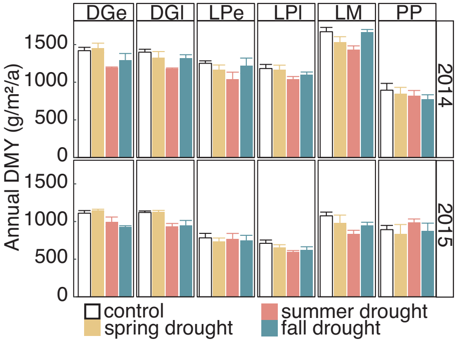

Fig. S2: Annual dry matter yield under rain-fed control and under the three seasonal drought treatments of the individual grasses in the years 2014 and 2015. Values are means ± se across four replicates.

Chapter 2

Higher drought resistance and physiological activity during reproductive spring growth in temperate grasses

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Abstract

Increasing alterations in the frequency and intensity of precipitation events are predicted for the near and far future. While effects on the productivity and the worldwide carbon cycle are well-examined, the seasonality of drought events has not been fully investigated yet. Here we tested, if the drought sensitivity of grasses varies throughout the growing season. Since the same duration of a drought period can produce different drought intensities, depending on a variety of environmental conditions (e.g. evaporative demand), comparing drought between seasons is difficult. To account for different drought intensities between the seasonal drought periods we analyzed the response variables in our experiment as continuous dependent variables and tested if differences in drought sensitivities of the plants can be attributed to the plants' developmental stage or its physiological activity. For the experiment we, exposed commonly used forage grasses to seasonal drought treatments that occurred either in spring, summer or fall in two consecutive years while measuring the corresponding soil water potential (Ψ_{soil}). Spring growth rates were most resistant to drought, while summer and fall growth rates were similarly sensitive to drought. Against our expectations growth rates of reproductive developmental stages were less affected by drought than those of vegetative stages in all tested grasses. Midday leaf water potential (midday Ψ_{Leaf}) and stomatal conductance (g_s) were least affected by spring drought, as well as carbon discrimination, compared to the other seasonal drought periods. With this our study shows that (i) not only the productivity, but also the physiological activity of the tested grasses is less sensitive to drought in

spring than in other seasons and (ii) that plants during reproductive growth may invest more in drought resistance than during vegetative growth.

Introduction

Climate change will lead to altered precipitation patterns (Orth *et al.*, 2016) and changes in the duration and intensity of drought events leading to extended drought periods (Seneviratne *et al.*, 2012; Trenberth *et al.*, 2013). These changes in the distribution and intensity of rainfall events within the growing season will impact aboveground biomass production (Swemmer *et al.*, 2007; Wu *et al.*, 2011; Reichstein *et al.*, 2013; Lei *et al.*, 2016).

Negative impacts of drought events on the productivity of ecosystems are a well-documented and common phenomenon (Gilgen & Buchmann, 2009; Hoover *et al.*, 2014; Grant *et al.*, 2014). However, it is unclear if in temperate ecosystems the impact of drought on plant productivity and other ecosystem functions differs in different parts of the growing season. In fact, only few studies exist to date that have directly assessed varying seasonal impacts of drought on ecosystems. Craine *et al.* (2012) found, for example, that the effect of a drought on the productivity of a temperate, humid grassland declined over the growing season with no impact of drought on grassland productivity in August. In a study conducted by Swemmer *et al.* (2007) temperate grassland annual productivity differed strongly between sites depending on precipitation pattern and timing and independent from precipitation amount. In a previous study by Hahn *et al.* (in prep.) we found that growth of temperate grasses, indeed, was differently affected by drought throughout the growing season. While high productive spring growth was least sensitive to drought, summer and fall growth was much more decreased by drought.

Seasonal differences in the responses of plant productivity to drought could be caused by a variety of mechanisms. Different drought responses throughout the growing season could be the result of the different plant developmental stages (PDS). Plants undergo several developmental stages throughout a growing season, simplified distinguishable in vegetative and reproductive growth. Several studies suggest that the productivity of plants, such as cereals, is most sensitive to drought in mid-reproductive stages while tillering during the vegetative phases is least affected by soil drought (Salter, 1967; O'Toole & Cruz, 1980; Siddique *et al.*, 2000). Osmotic adjustment (OA) is a characteristic that is able to develop in response to water stress. Via an increase of osmotically active substances within metabolically active cells a more negative osmotic potential is created, which can improve cell hydration or maintain cell turgor. OA, thus, helps plants to survive longer and maintain metabolic processes under drought. In crop cultivars, for example, OA can improve growth and yield under drought (Sanders & Arndt, 2012). For sorghum OA has been studied quite extensively (Morgan, 1983; Santamaria *et al.*, 1986, 1990) and has been found to depend on a variety of factors, including the stage of development (Turner & Jones, 1980). However, OA is an inducible and not inherent characteristic and yet knowledge for temperate grasses and how different plant developmental stages may develop OA is missing. While the drought sensitivity of different stages is well examined for a variety of crops (wheat: Moliboga (1927); maize: Robins & Domingo (1953); Denmead & Shaw (1960); barley: Aspinall *et al.* (1964); oats: Seelhorst (1911)), drought impacts on the productivity of different developmental stages of perennial grasses have not yet been assessed. This is surprising given that grasslands are a key model ecosystem for the assessment of drought impacts on plant productivity and ecosystem functions.

Another critical factor shaping plant responses to drought events is the physiological performance of plants. Photosynthesis is linked to processes like leaf water status and leaf gas exchange which are known to be very sensitive to environmental conditions, such as air temperature, radiation and water availability (Signarbieux & Feller, 2012). Leaf water potential (Ψ_{Leaf}) is physically associated with the stomatal aperture. Hence, mechanisms like stomatal closure enable plants to survive drought through saving water. As a consequence thereof productivity and yield are most certainly reduced (Turner & Jones, 1980). However, to release plants from water stress irrigation has only little impact. Instead a reduction of atmospheric evaporative demand is required to decrease water stress in plants (Jackson, 1974). Since environmental conditions (including vapor pressure deficit (VPD)) differ throughout the growing season, changes in the response of the vegetation to a drought event may be attributed to altering physiological sensitivities of plants to drought in different seasons.

It could also be that not the sensitivity of the vegetation, but in contrast, the severity of drought events systematically differs throughout the growing season. This is, because drought on the one side is the result of a low soil moisture content or soil water potential (Ψ_{Soil}), which gives an adequate measure for the drought impact on an ecosystem (Vicca *et al.*, 2012). Generally, in temperate ecosystem the soil is water-saturated at the beginning of the growing season in spring, while soil moisture decreases towards summer and recovers again towards the end of the growing season (Brinkmann *et al.*, 2016; Dietrich *et al.*, 2018). Thus, the absence of rain during spring causes a much slower drying of the soil than equal levels of missing precipitation in summer, where soil moisture is already low. This is supported by a much lower evaporative demand of the atmosphere and lower ecosystem transpiration due to smaller leaf area indices (LAI) in spring. Thus, in drought experiments, where only precipitation is manipulated, it is possible that solely the drought intensity, but not the drought response of the ecosystem is altered throughout the growing season. Equally important, however, is the VPD, which determines the atmospheric demand for moisture, and thus, plant water use also determines the intensity of a drought event. VPD is more likely to be high in summer leading to higher evapotranspiration rates by the plants than in spring (De Boeck *et al.*, 2010, 2011).

In a previous study we investigated the effects of drought events occurring at different times of the growing season on the productivity of temperate grasses. While growth was affected by drought events in every season, surprisingly most productive spring growth was least susceptible to water scarcity (Hahn *et al.*, in prep.). However, the underlying mechanisms leading to growth rates in spring being most resistant to drought remain unclear. Thus, we were interested in understanding *why* drought effects on the growth of temperate grasses differ between seasons. Specifically, we investigated

i. if varying drought sensitivites of temperate grasses across the growing season is the result of different experimentally induced drought intensities,

ii. if the drought response at a given drought intensity depends on the plant developmental stage,

iii. if the drought stress experienced by the plants physiologically (expressed as midday leaf water potential (midday Ψ_{Leaf}) and stomatal closure (g_s, $\delta^{13}C$)) at a given drought intensity differs between the seasons.

To enable the generality of our findings we studied these three questions using up to six different temperate grasses that are commonly used in agricultural practice.

Methods

Research site

The experiment was carried out for two years from 2014 until 2015 near Zurich, Switzerland (47°26'N, 8°31'E, altitude: ~490m a.s.l., mean annual temperature: 9.4°C, mean annual precipitation: 1031 mm). Monocultures of six widely used forage grasses were sown in August 2013 on 196 3 × 5 m plots.: *Lolium perenne* L. 'Artesia' (LPe), *Lolium perenne* L. 'Elgon' (LPl), *Dactylis glomerata* L. 'Barexcel' (DGe), *Dactylis glomerata* L. 'Beluga' (DGl), *Lolium multiflorum* Lam. var *italicum* Beck 'Midas' (LM), *Poa pratensis* L. 'Lato' (PP). At the beginning of each growing season the soil was fertilized with 39 kg P/ha, 228 kg K/ha and 35 kg Mg/ha. In addition, all plots received the same amount of solid N fertilizer; 80 kg N/ha at the beginning of the growing season and 40 kg/ha after each of the first five cuts (5 × 40 kg N/ha).

Experimental design

All grasses were subject to four treatments: one rain-fed control treatment and three seasonal drought treatments (spring, summer, fall) to cover all developmental stages. The drought treatments were implemented via rain-out shelters and differed in timing and duration between two parallel running experiments (series A and B, see Fig. 1). In series A, rainfall was excluded for approx. ten weeks in every season (Table 1) on the respective treatment plots in both years. In 2014, series B was treated similarly to series A, but with a two-week offset (delay) in the seasonal drought treatments. In 2015, the drought periods in series B were expanded to 15 weeks and only implemented in summer and fall (Fig. 1).

The tunnel-shaped shelters, which completely excluded rainfall, consisted of steel frames (3 × 5.5 m, height 140 cm) covered with transparent and UV radiation transmissible greenhouse foil (Lumisol clear, 200my, Hortuna AG, Winikon, Switzerland). Proper air circulation was allowed by ventilation openings of 35 cm over the entire length at the top and bottom of both sides of the shelters and additionally by open ends on the shorter sides. These shelters had previously been successfully tested in other grassland-drought experiments (Hofer *et al.*, 2016, 2017).

Controls were subject to the natural precipitation regime (see Hahn *et al.*, unpublished data). However, when soil water potential (Ψ_{Soil}) sank below -0.5 MPa due to naturally dry conditions, all control plots were additionally watered with 300 l of water (20 mm per plot). Watering happened once on June 16th and 17th 2014 and three times in 2015 (7./8.7., 14./15.7., 11./12.8.).

Environmental measurements

Air temperature and relative humidity were surveyed with VP-3 humidity, temperature and vapor pressure sensors (Decagon Devices, Inc., Pullman WA 99136, USA). Measurements were conducted in control as well as treatment plots under the rainout shelters (n=2). The national meteorological service stations that were in immediate proximity of the research site (average of the two surrounding meteorological stations Zurich Affoltern in 1.4 km distance and Zurich Kloten in 4.5 km distance) provided additional information regarding precipitation and evapotranspiration. Soil water potential sensors (MPS-2 dielectric water potential sensors, Decagon Devices, Inc., Pullman WA 99136, USA) were installed in 10 cm depth and recorded on an hourly basis. The 64 sensors in 2014 and 56 sensors in 2015 were evenly distributed over the field and treatments. Daily means of all measurements were calculated from the data delivered by the sensors per series and treatment, but across species since no species-specific alterations in Ψ_{Soil} were expected (Hoekstra *et al.*, 2014) or measured (n=8).

Table 1: Overview of the timing of the seasonal treatments in 2014 and 2015 for both series.

	Spring treatment	Summer treatment	Fall treatment
2014			
Serie A	03-12 – 05-21	05-21 – 07-30	07-30 – 10-17
Serie B	03-12 – 06-04	06-04 – 08-13	08-13 – 10-31
2015			
Serie A	03-11 – 05-20	05-20 – 07-29	07-29 – 10-07
Serie B	----------	03-11 – 06-17	06-17 – 09-30

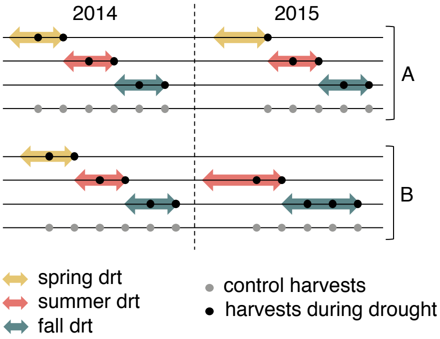

Fig. 1: Experimental Design; the experiment lasted for two consecutive years (2014, 2015) with twelve harvests (•) evenly distributed over both growing seasons in each treatment. Arrows indicate the duration of each drought treatment (ten to 15 weeks). Each treatment was replicated four times and three times in series A and B, respectively.

Developmental status of the sown grasses was examined via visual classification into different stages. Plants were considered to be in a vegetative stage during tillering. Generative stages contained sprouting, panicle development and flowering.

Harvests

In 2014, aboveground biomass was harvested six times per year at a five-week interval with two cuts per seasonal drought treatment (Fig. 1). The harvests matched with the treatments and occurred five and ten weeks after the installation of the rainout shelters. In 2015, aboveground biomass of series A was harvested similarly to 2014. Series B, however, was harvested six times per year at a five-week interval with three cuts in the summer and fall treatment each, that matched with the treatments and occurred five, ten and 15 weeks after the installation of the rainout shelters. Aboveground biomass was harvested at 7 cm height from a central strip of 5 × 1.5 m using an experimental plots harvester (Hege 212, Wintersteiger AG 4910 Ried/I., Austria). Dry matter yield of each plot was determined by assessing dry weight – fresh weight ratios of the harvested biomass. For this a biomass subsample was collected for each plot and the fresh and dry weight (dried at 60°C for 48 h) were determined. After the harvest of the aboveground biomass in the central strip of a plot, the remaining standing biomass in a plot was mowed 7 cm above the ground and removed.

Determining drought impacts on growth

For comparing grassland productivity of the different developmental stages and seasons across the two years we standardized the productivity that occurred in-between two harvest periods for growth related temperature effects and calculated temperature corrected growth rates (DMYTsum, see Menzi *et al.* (1991)). To this aim, we calculated temperature sums of daily mean air temperature above a base temperature of 5°C (Tsum) under the rainout shelters for the respective seasonal treatment and in free air for the control for each regrowth period (i.e. 5 weeks prior to harvest). Dry matter yield (DMY) of a given harvest was then divided by the temperature sum of the corresponding time period and treatment to obtain temperature-weighted growth rates:

$$\mathrm{DMYTsum} = \mathrm{DMY}(\mathrm{g/m^2})/\mathrm{Tsum}(^\circ\mathrm{C}).$$

To identify drought effects on aboveground growth relative to the control, we first calculated the response ratio (R) between temperature-weighted growth rates in the drought treatment and control:

$$\mathrm{R} = \mathrm{DMYTsum(drt)}/\mathrm{DMYsum(ctr)}$$

and calculated the relative change of growth (RCG) as:

$$\mathrm{RCG} = 100\times(\mathrm{R}-1).$$

The corresponding Ψ_{soil} for each regrowth period to the harvested DMYTsum was used to calculate the median of Ψ_{soil}, giving a measure for the severity of drought in this period.

Physiological measurements

In 2015, measurements of midday Ψ_{Leaf} and stomatal conductance (g_s) were conducted on four of the six grasses, namely both *D. glomerata* and both *L. perenne* grasses. Midday Ψ_{Leaf} was measured throughout the growing season on 17 days around the early afternoon at peak Ψ_{Leaf} (pre-tested in the course of previous days). Leaves were cut neatly at their base and immediately measured. All measurements were done according to Scholander *et al.* (1964) using a PMS 600 pressure chamber (PMS Instrument Company, Albany, OR 97322, USA). g_s was measured along with midday Ψ_{Leaf} using a SC-1 Leaf Porometer (Decagon Devices, Inc., Pullman WA 99136, USA) on the underside of the leaves. Since g_s measurements of grass blades are not common, the aperture of the leaf porometer was larger in diameter than most grass blades. According to the company's advice, several grass blades were put together side by side and fixed together with the help of foam-covered clothespins to cover the aperture's diameter completely.

$\delta^{13}C$ values of plants

As a measure for the physiological impact of drought during the different developmental stages of the tested grasses, the carbon isotope composition (i.e. $\delta^{13}C$ values) of the harvested plant material was measured (Dawson & Siegwolf, 2007). The carbon isotope composition of plant tissue describes the ratio of leaf internal to atmospheric CO_2 pressure. Leaf internal CO_2 pressures are determined by net assimilation and g_s. Thus, the $\delta^{13}C$ values of newly assimilated plant tissue can indicate declining g_s (Farquhar *et al.*, 1989). The analysis of the plant tissue for $\delta^{13}C$ was done with a Delta V isotope ratio mass spectrometer coupled to a flash EA (Thermo Scientific, Bremen, Germany). For this, the dried plant material was milled into fine powder using a swing mill (Retsch MM400, Retsch GmbH, Haan, Germany) and 3 to 4 mg of the powder was weighed and placed into silver capsules. The carbon isotope composition of the plant material is indicated as the $^{13}C/^{12}C$ ratio of the plant material relative to an international standard (Vienna Pee Dee Belemnite) (Condon *et al.*, 2002).

Data analysis

Instead of testing the response variables in a categorial manner we analyzed changes in DMYTsum as a function of Ψ_{soil} in the different treatment periods using linear mixed-effects regression (Pinheiro & Bates, 2000). The data included measurements of series A and B from 2014 but only series A from 2015, since the classification of seasons in series B was divergent. The log response ratio (natural logarithm) of temperature-weighted growth rate (ln(R) = ln(DMYTsum(drt)/DMYTsum(ctr)) was regressed against the fixed variables soil water potential (Ψ_{soil}; continuous), season (factor of three levels: spring, summer, fall), and grass (factor of six levels), including all interactions. To account for repeated measurements over the two years, plot was specified as a random factor and a temporal compound symmetry correlation structure was imposed on the residuals (thereby accounting for potential correlation of residuals over time). Moreover, inspection of residuals revealed clear differences in their variance among seasons, and therefore the residual variance parameter was defined as $\mathrm{Var}(e_j) = \sigma^2\delta_j^2$, with δ being a ratio to represent j variances, one for each of three seasons j (Pinheiro & Bates, 2000). The R^2 of explained variance of fixed effects was computed following Nakagawa & Schielzeth (2013).

Changes in DMYTsum depending on the developmental stage was analyzed in a similar way. Here, separate liner mixed-effects models were run for each of the grasses and data included both series A and B from both years 2014 and 2015. The log response ratio ln(R) (as defined) was regressed against the fixed variables Ψ_{soil} and plant developmental stage (factor of two levels), including their interaction. The model further included plot as a random factor and a temporal compound symmetry correlation structure, and the residual term was defined as $Var(e_j) = \sigma^2\delta_j^2$, with δ being a ratio to represent a variance for each of the two developmental stages j.

All statistical analyses were conducted with R, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria, 2018), while all graphics were done with the package *ggplot2*, version 2.1.0 (Wickham, 2016). We assumed a 5 % level of significance.

Results

The temperature-weighted growth rates of the grasses declined with decreasing Ψ_{soil} in all three drought treatments (Table 2, Fig. 2). The magnitude of the growth response differed, however, between the seasons a drought occurred in (Table 2). In general, temperature-weighted growth rates were less sensitive to drought in spring, as compared to growth rates of the grasses in summer and fall (Fig. 2). As such, the % reduction in temperature-weighted growth relative to the control was consistently less severe at any given Ψ_{soil} in spring than in summer or fall.

Table 2: Summary of analysis for the effects of soil water potential (Ψ_{soil}), season of drought, grass, and their interactions on the log response ratio of temperature-weighted growth rates (ln(DMYTsum(drt)/DMYTsum(ctr))) during drought. The inference (F- and p-values) and the R^2 refers to the fixed effects from the linear mixed model. df_{num}: degrees of freedom term, df_{den}: degrees of freedom of error.

Effect	df_{num}	df_{den}	F-value	p
Ψ_{soil}	1	228	240.2	<0.001
Season	2	108	35.7	<0.001
Grass	5	108	1.5	0.185
Ψ_{soil} × Season	2	228	2.3	0.101
Ψ_{soil} × Grass	5	228	5.7	<0.001
Season × Grass	10	108	0.6	0.784
Ψ_{soil} × Season × Grass	10	228	1.2	0.270
R^2: 0.44				

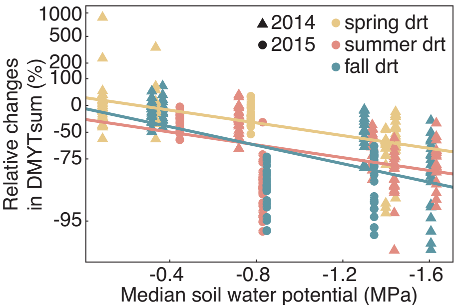

Fig. 2: Relative change of growth under drought compared to the control (RCG; %, displayed on log-scale) with increasing soil water scarcity (expressed as the median of the soil water potential (Ψ_{soil}) of the respective growth period). Trend lines are based on linear mixed-effects regression.

To test if different drought sensitivities across a growing season can be explained by the phenology of the investigated plants, we compared the drought responses of the investigated grasses depending on their plant developmental stage. In contrast to our expectation, growth rates of vegetative plant developmental stages were more reduced than those of reproductive ones in every grass at any given Ψ_{soil} (Fig. 3). This was consistent for all six tested grasses. In addition, both *L. perenne* grasses and the late-flo-

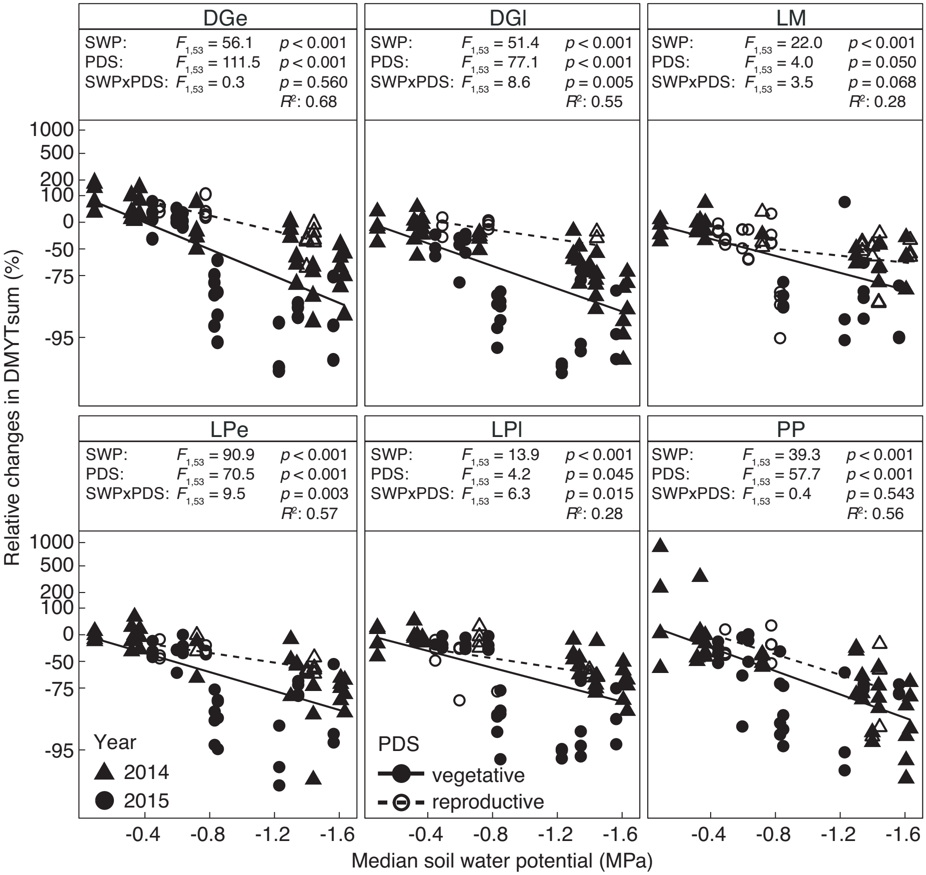

Fig. 3: Relative change of growth under drought compared to the control (RCG; %, displayed on log-scale) of six grasses with increasing soil water scarcity (expressed as the median of the soil water potential (Ψ_{soil}) of the respective growth period). Panels include the summaries of analyses for the effects of soil water potential (Ψ_{soil}; SWP), plant developmental stage (PDS), and their interactions on the log response ratio of temperature-weighted growth rates (see methods). Trend lines, the inference (*F*- and *p*-values) and the R^2 refers to the fixed effects from the fixed effects of the models.

wering *D. glomerata* grass (DGl) showed increasing sensitivities of vegetative stages with decreasing Ψ_{soil} as compared to their reproductive stages (Fig. 3; SWP × PDS $p<0.05$).

The other grasses showed no such interaction between plant developmental stage and increasing drought (Fig. 3).

We assessed if the physiologically experienced drought stress (i.e. midday Ψ_{Leaf}) differed for a given Ψ_{soil} across the growing season. We found that under conditions with less negative Ψ_{soil} plants showed higher midday Ψ_{Leaf} in spring than in summer or fall (Fig. 4a). However, with increasing drought intensity midday Ψ_{Leaf} converged in all seasons to a similar low value below -2 MPa for every of the four tested grasses (Fig. 4a). We found similar patterns when we used VPD as predicting variable. Again, at a given VPD plants showed less negative midday Ψ_{Leaf} as compared to plants in the summer or fall (Fig. 4b). With increasing VPD initial midday Ψ_{Leaf} differences between the seasons were diminished (Fig. 4b).

Complementary to midday Ψ_{Leaf}, we assessed if midday g_s (as a further measure for physiologically experienced drought) differed for a given Ψ_{soil} across the growing season. g_s was higher in both *D. glomerata* grasses in spring than in summer or fall at any given Ψ_{soil} (Fig. 5a). Both *L. perenne* grasses, in contrast, showed similar g_s values in all seasons at a given Ψ_{soil} (Fig. 5a). However, g_s decreased with increasing drought intensity (decreasing Ψ_{soil}) and converged to a similar low value near stomatal closure in all seasons and grasses (Fig. 5a). When using VPD as predicting variable for g_s we found similar patterns. In *D. glomarata* grasses, at a given Ψ_{soil} g_s was higher in spring than in summer or fall (Fig. 5b). For *L. perenne* grasses this was also observed, but much less pronounced (Fig. 5b). Similar to midday Ψ_{Leaf} g_s was decreased with increasing drought

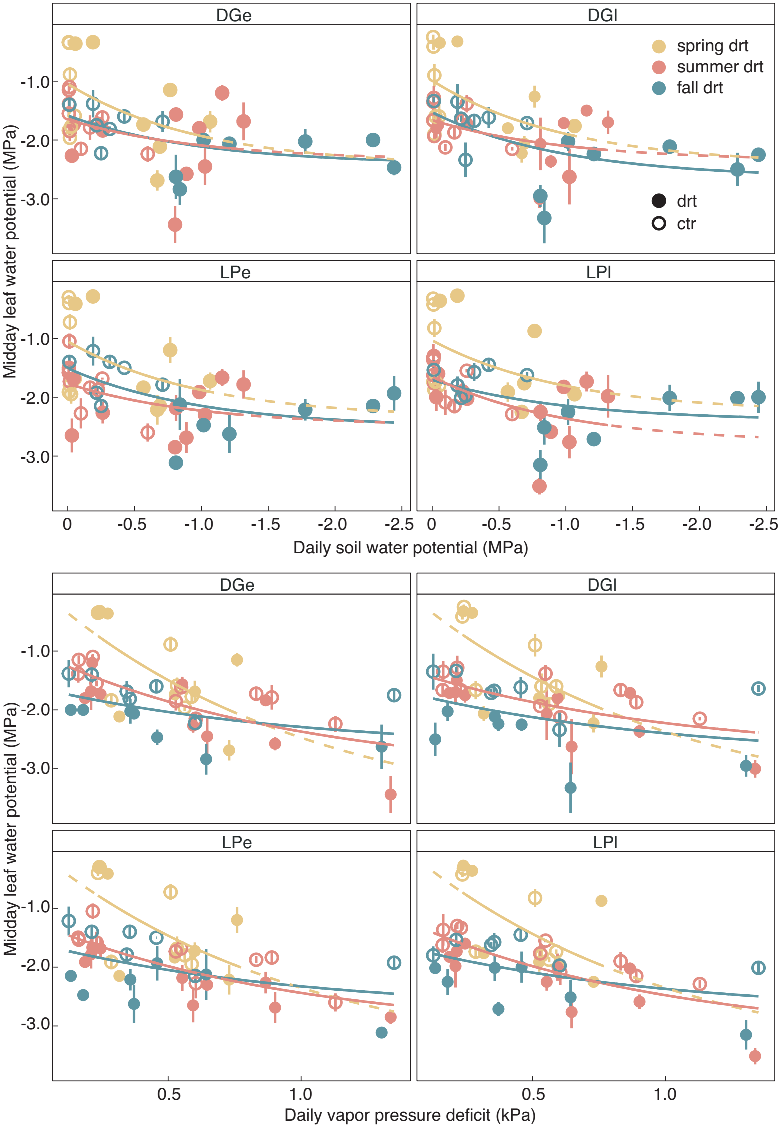

Fig. 4: Drought stress experienced (expressed as midday leaf water potential (midday Ψ_{Leaf})) by grasses with increasing soil water scarcity (expressed as daily soil water potential (Ψ_{soil}); upper four panels) and increasing vapor pressure deficit (VPD; bottom four panels) in 2015. Values are means ± se of four replicates of series A and three replicates of series B for both *D. glomerata* (DGe, DGl) and *L. perenne* (LPe, LPl) grasses. Trend lines are based on exponential regression and are dotted for values beyond the measurement range. Hollow shapes represent measurements of control plots, filled shapes represent measurements of treatment plots.

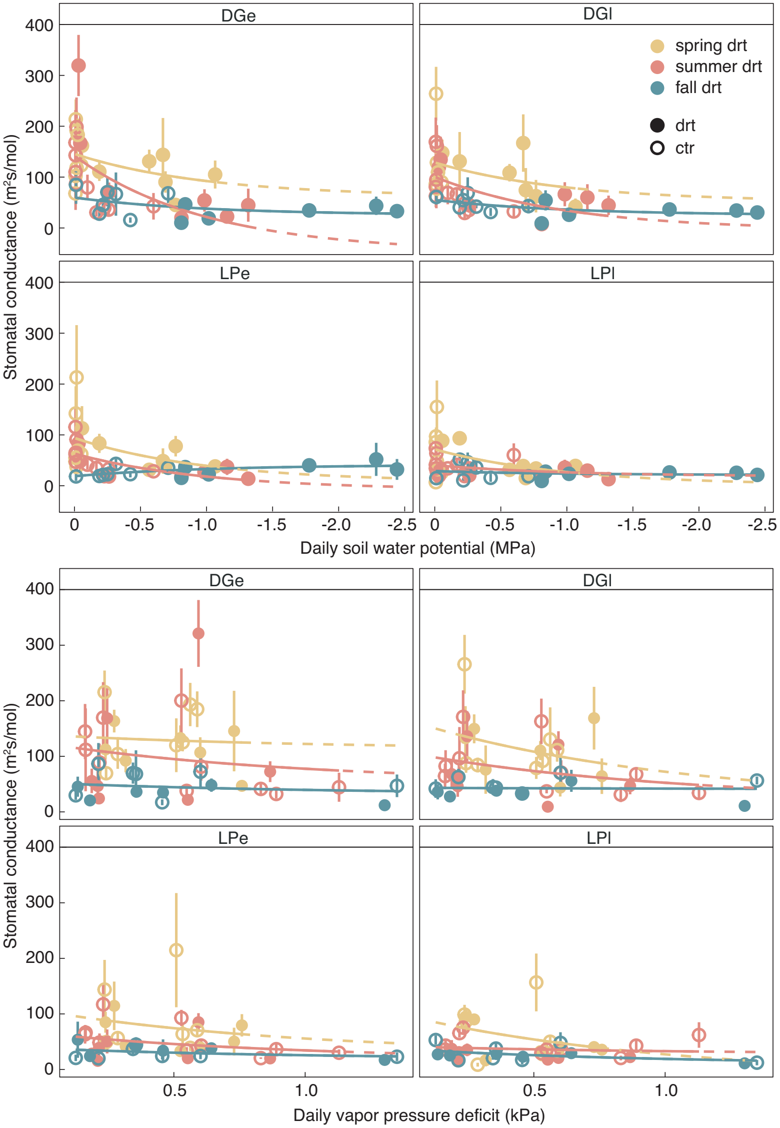

Fig. 5: Midday stomatal conductance (midday g_s) of grasses with increasing soil water scarcity (expressed as daily soil water potential (Ψ_{soil}); upper four panels) and increasing vapor pressure deficit (VPD; bottom four panels) in 2015. Values are means ± se of four replicates of series A and three replicates of series B for both *D. glomerata* (DGe, DGl) and *L. perenne* (LPe, LPl) grasses. Trend lines are based on exponential regression and are dotted for values beyond the measurement range. Hollow shapes represent measurements of control plots, filled shapes represent measurements of treatment plots.

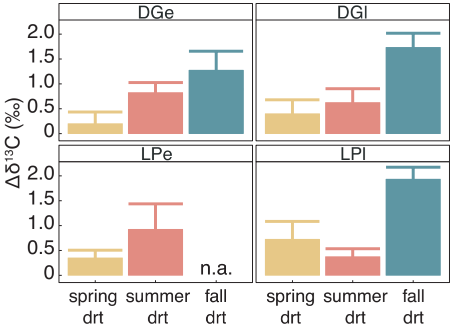

Fig. 6: Drought stress experienced (expressed as the difference of ^{13}C values (‰) of plant material of the control and treatments ($\Delta\delta^{13}C=\delta^{13}C_{Treatment}-\delta^{13}C_{Control}$)) depending on the season of drought in 2015. Values are means ± se of four replicates of series A for both *D. glomerata* (DGe, DGl) and *L. perenne* (LPe, LPl) grasses at the end of each drought treatment. Panels divide the grasses.

intensity in all four grasses (Fig. 5b). In general, VPD in spring did not exceed values of 0.8 kPa, while VPD in summer and fall reached values up to 1.5 kPa (Fig. 4b, 5b).

To assess temporally integrated physiological drought responses of the grasses in different times of the growing season, we measured ^{13}C values in the plant tissue of treatment and control plants and calculated the treatment effect as the difference between $\delta^{13}C$ between the treatment and the corresponding control (i.e. $\Delta\delta^{13}C$). $\Delta\delta^{13}C$ values were smallest in spring and highest in fall for both *D. glomerata* grasses (Fig. 6), suggesting a higher physiological drought sensitivity in the summer and, in particular, in the fall compared to the spring. $\delta^{13}C$ in the early-flowering *L. perenne* grasses (LPe) was also least reduced under spring drought and more reduced under summer drought, but no value for the fall exists. This is caused by the extremely reduced productivity of the early-flowering *L. perenne* grass (LPe) in fall, that did not allow $\delta^{13}C$ analysis (Fig. 6). In the late-flowering *L. perenne* grass (LPl) drought in summer lead to the lowest differences in $\delta^{13}C$ between the control and the treatment plant material, while differences were

Table 3: Median of daily soil water potential (Ψ_{soil}) and vapor pressure deficit (VPD) during the different drought periods ± sd.

	spring	summer	fall
median Ψ_{soil} (MPa)	-0.7 ± 0.6	-0.8 ± 0.6	-1.1 ± 0.6
median VPD (kPa)	0.4 ± 0.2	0.5 ± 0.4	0.4 ± 0.4

much higher in the fall (Fig. 6). This suggests a higher physiological drought sensitivity, particularly, in the fall compared to spring and summer.

Discussion

Comparing the impacts of drought events that occur in different times of the growing season on the functioning of temperate ecosystems is experimentally challenging. This is because defined and comparable levels of stress are difficult to produce in large outdoor experiments throughout a growing season. For practical reasons, most drought experiments in ecology and ecosystem science manipulate precipitation input only (Hanson, 2000; Beier *et al.*, 2012; Kreyling *et al.*, 2017) and the severity of a drought treatment is typically manipulated by the duration of the rainfall exclusion (Vicca *et al.*, 2012). However, the severity of a drought event depends on multiple variables next to precipitation input. In particular, this is (i) the residual moisture available in the soil at the beginning of the drought treatment, (ii) the evaporative demand of the atmosphere, and (iii) the amount of water lost from the ecosystem through evapotranspiration during the drought period. As such, drought treatments at different times in the growing season, that are of identical duration, do not necessarily induce identical drought stress levels on the ecosystems, and thus, need to be compared with caution (Table 3). To avoid the problem of having installed drought treatments that are identical in length, but possibly differ in their severity, we analyzed the response variables that we determined from the different seasonal drought treatments in our experiment as continuous dependent variables. As such, we were able to compare the drought response of the response variables at a given Ψ_{soil} or VPD across two growing seasons that we investigated.

Our analysis revealed clear seasonal differences in the drought sensitivity of the growth rates of the six grasses (Table 2, Fig. 2). In contrast to our expectations, we found spring growth to be most resistant to drought, while growth in summer and fall was more sensitive to declining soil moisture (see also Hahn *et al.*, in prep.). Interestingly, grasses that were in a reproductive stage were also more resistant to drought than grasses in a vegetative state. We further found that at a given Ψ_{soil} foliar midday water potentials were less negative in spring than foliar midday water potentials in summer or fall. The same was true for a given VPD. Less negative foliar water potentials for a given Ψ_{soil} or VPD in spring compared to summer or fall cannot be explained by reduced, and thus, more conservative water use of the plants. In contrast, for a given Ψ_{soil} or VPD we find slightly higher spring time g_s (indicated by direct gas-exchange measurements) as well as stable carbon isotopes values. Our data, thus, suggests that not only the productivity, but also the physiological activity of the investigated grasses is less drought sensitive in spring compared to the summer or fall.

Based on Salter (1967), who found a variety of crops to be most sensitive during reproductive growth we expected similar results in our study. Contrary to our expectations, growth rates of reproductive developmental stages were least sensitive to drought. In some grasses reproductive stages were even less affected by increasing drought intensity than vegetative stages (Fig. 3; SWP × PDS $p<0.05$). Water availability is one of the main factors for plant growth, both vegetative and reproductive. Since fundamental processes within the plant like maintenance of cell turgor or protein synthesis etc. depend on water expansive growth is strongly affected by water shortage (Sánchez *et al.*, 1998; Sanders & Arndt, 2012). However, reproductive developmental stages are phases where a plant establishes reproductive organs and does not provide growth processes in the narrower sense. Drought events during reproductive growth, thus, can affect grain yields during grain filling (Fischer, 1979) or reduce the number of florets during floret initiation (Chinnici & Peterson, 1979), but expansive growth as such is less affected (Bradford & Hsiao, 1982). For plants reproductive developmental stages, where generative organs are developed, are of high importance to secure reproductive success and, hence, survival. One reason for reproductive stages being less sensitive to drought, thus, can be that plants invest in mechanisms that ensure drought resistance during that important phase of their life cycle. Plants investing in root growth during drought in generative growth stages, for example, may increase the access to deeper soil layers with higher soil water availability, thus, ensuring the proper development of reproductive organs while vegetative growth stages still depend on the scarce soil water availability of topsoil layers. For the tested grasses in this study, however, this process can largely be eliminated, since investment in root growth could not be detected in any season under drought (Hahn *et al.*, in prep.). Alternatively, the investment in OA of plants during drought-exposed reproductive growth can increase the drought resistance by maintaining cell turgor and improving cell hydration (see introduction) to sustain important metabolic processes, including the formation of reproductive plant organs. Consequently, in our study vegetative plant developmental stages being more sensitive to drought than reproductive stages may be explained by increased drought resistance during plant reproduction.

To evaluate the mechanisms of reduced springtime drought sensitivity, we assessed key physiological variables. During spring growth (where most grasses were in reproductive growth stages) midday Ψ_{Leaf} at a given drought intensity was less negative than during vegetative growth throughout the rest of the growing season. Moreover, high levels of g_s and low levels of $\Delta\delta^{13}C$ in spring compared to summer and fall indicate low levels of stomatal closure in response to drought in spring. Hence, during spring the tested grasses were much more physiologically active even under drought than in summer or fall. The obvious higher physiological activity of the plants during spring drought, apparently, plays a crucial part in the growth response of the plants, since growth rates were less sensitive to drought in spring. Again, as stated above already, mechanisms like investment in root growth or OA may explain the higher physiological activity of the plants under spring drought. We previously showed that the tested grasses in this experiment did not invest in root growth during drought, at least not in terms of root biomass (Hahn *et al.*, in prep.). However, root biomass may not be a comprehensive measure if we consider (i) that root distribution along the soil gradient is not automatically reflected by root biomass (Herndl *et al.*, 2011) and (ii) the activity of the roots is not necessarily equivalent with root biomass (Kulmatiski & Beard, 2013). Thus, higher rates of physiological activity of the plants under spring drought may be due to alterations in the rooting system. Although we have no evidence for OA in this study, OA is a process that is widespread along grasses (Morgan, 1984) and Morgan (1983) found increased grain yield in wheat lines with high osmoregulation compared to lines with low osmoregulation, suggesting that reproductive stages profit from OA. Thus, reproductive growth stages in spring may develop less negative midday Ψ_{Leaf} due to OA although their water consumption is equal to vegetative growth stages during summer or fall. Moreover, photosynthesis and carbon assimilation can be influenced by metabolic alterations under drought that are not reflected in midday Ψ_{Leaf} or g_s (Medrano *et al.*, 2002). Signarbieux & Feller (2011) detected non-stomatal limitation in the form of a reduced carboxylation velocity of Rubisco (which is highly sensitive to water shortage) in grasslands throughout Switzerland under drought. This may explain strong differences in δ^{13}C between control and drought affected plant material during fall although stomatal closure was increasing towards the end of the growing season. Signarbieux & Feller (2011) also detected species specific differences in the ratio of stomatal to non-stomatal limitation, indicating why differences in g_s and carbon discrimination between *L. perenne* and *D. glomerata* emerged.

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Chapter 3

Changes in forage quality under seasonal drought

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Abstract

While changes in the precipitation pattern are known to increase the frequency and intensity of drought events, seasonal differences of drought events on the ecosystem functioning are not conclusively assessed. While drought events are known to reduce grassland productivity in most cases, knowledge about the effects on forage quality of fodder grasses is less investigated, especially when it comes to seasonal differences between drought events. Here we tested, how drought events influence the quality of forage plants throughout the growing season and if drought events occurring at different times of the growing season lead to different plant responses. In addition, we tested for legacy effects after drought-release potentially altering forage quality post-drought. Moreover, we tested for plant functional type specific differences between grasses and one legume species. While forage quality of grasses was not affected under seasonal drought, the legume species showed reduced nitrogen (N) content, reduced digestible organic matter (DOM) and net energy of lactation (NEL) due to drought. In the post-drought period the grasses profited from the drought release with an increased N content in the plant tissue, while the nutritive value of the legume remained unaffected after the release from drought. Although seasonal differences in forage quality could be detected throughout the growing season, the effect of drought events occurring at different times of the growing season on quality parameters were diverse and showed no clear pattern. With this our study shows that i) forage quality, indeed, differs throughout the growing season, ii) immediate drought events rather reduce than increase forage

quality, iii) legacy effects of drought enhance forage quality and iv) that forage quality changes due to drought strongly depend on the plant functional type.

Introduction

Due to global warming changes in precipitation, including the frequency and intensity of drought events, are predicted to impact major ecosystem processes (Trenberth *et al.*, 2003; Seneviratne *et al.*, 2012). Many studies so far have focused on impacts of drought events on the productivity of ecosystems (Gilgen & Buchmann, 2009; Hoover *et al.*, 2014). However, ecosystem responses to water scarcity depend on a variety of factors, including the ecosystem type (Byrne *et al.*, 2013; Wolf *et al.*, 2013), the biodiversity (Kahmen *et al.*, 2005; Isbell *et al.*, 2015; Wagg *et al.*, 2017) or the intensity of a drought event (Vicca *et al.*, 2012). A loss of productivity due to drought, nonetheless, is one of the main observations that all studies have in common (Wu *et al.*, 2011).

Since existing studies have focused on drought effects on productivity, possible effects of drought events on other parameters have been widely neglected, in particular, when it comes to the quality of produced forage under drought. Forage quality depends on nutrient concentration, such as nitrogen (N), non-structural carbohydrates (NSC), structural carbohydrates (including neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL)) and anorganic components like crude ash (CA). Moreover, forage quality includes parameters like digestibility of organic matter (DOM) and net energy of lactation (NEL). In previous studies, drought effects on forage quality were diverse. While some studies proved crude protein (CP) content, which strongly correlates with N content, to be increased under drought (Grant *et al.*, 2014;

Dumont *et al.*, 2015), others found no immediate effect of drought on CP or N content (Küchenmeister *et al.*, 2014).

Although temperate ecosystems show a distinct seasonality, where grasses undergo a variety of phenological, physiological and morphological stages throughout their life cycle (Voigtländer & Jacob, 1987), existing studies have neglected possible seasonal effects of drought events, particularly when it comes to the quality of produced forage. Although it is known that, for example, digestible organic matter decreases within the growing season in some species (Schubiger *et al.*, 1997; Smith *et al.*, 1998), the knowledge about how seasonal drought events affect forage quality parameters is sparse.

In a previous study, we investigated the effects of seasonal drought on productivity of temperate grasses (Hahn *et al.*, in prep.). While we found growth to be reduced in every season under drought, we also found growth to be differently sensitive to drought, depending on the season a drought occurred. To expand the knowledge about seasonal sensitivities of grasslands, we were also interested in how forage quality is affected. For a holistic view of forage quality, a selection of several parameters is recommended (Grant *et al.*, 2014). In this study, thus, we focused on the forage components N and CA. We further calculated DOM and NEL as parameters directly relevant for agricultural farming. Specifically, we were interested in

i. how forage quality alters throughout the growing season,

ii. how forage quality is affected by drought events that occur in different times of the growing season,

iii. if changes in forage quality under drought increase with increasing drought intensity, and

iv. if drought legacy effects altering the quality of forage can be detected after drought-release.

To be able to affiliate plant responses to changes in metabolic processes within the plants or to an altered nutrient uptake from the soil of the plants we compared different grasses and one legume species, both commonly and widely used in agricultural practice.

Methods

Research site

The experiment was conducted in the years 2014 and 2015 on a field site near Zurich, Switzerland (47°26'N, 8°31'E, altitude: 490 m a.s.l., mean annual temperature: 9.4°C, mean annual precipitation: 1031 mm). For the experiment monocultures of six forage grasses, widely used for forage production, and one legume were sown in August 2013 on 168 3 × 5m plots.: *Lolium perenne* L. 'Artesia' (LPe), *Lolium perenne* L. 'Elgon' (LPl), *Dactylis glomerata* L. 'Barexcel' (DGe), *Dactylis glomerata* L. 'Beluga' (DGl), *Lolium multiflorum* Lam. var *italicum* Beck 'Midas' (LM), *Poa pratensis* L. 'Lato' (PP) and *Trifolium repens* L. 'Bombus' (TR). Phosphorous, potassium and manganese were applied following Swiss local fertilization recommendations for intensely managed grasslands at the beginning

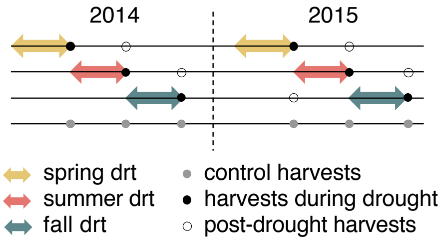

Fig. 1: Experimental design; experiment lasted two consecutive years (2014, 2015). Arrows indicate the duration of each drought treatment (ten weeks). Analysis for forage quality happened at the end of each drought treatment (every ten weeks in both growing seasons). Each treatment was replicated four times.

of each growing season (39 kg P/ha, 228 kg K/ha, 35 kg Mg/ha). In addition, all grass plots received the same amount of N fertilizer (280 kg N/ha, divided into six applications per year). The solid N fertilizer was applied at the beginning of the growing season (80 kg N/ha) and after each of the first five cuts (40 kg N/ha each time).

Experimental design

The tested grasses were subject to four treatments: one control and three seasonal drought treatments (spring, summer, fall) during each growing season (see Table 1, Fig. 1). A drought treatment lasted for ten weeks and was simulated using rainout shelters that excluded rainfall completely on the treatment plots. The tunnel-shaped rainout shelters consisted of steel frames (3 × 5.5 m, height: 140 cm) that were covered with transparent and UV radiation transmissible greenhouse foil (Lumisol clear, 200 my, Hortuna AG, Winikon, Switzerland). For air circulation, the shelters were open on both opposing short ends and had ventilation openings of 35 cm height over the entire length at the top and the bottom at both long sides. These shelters had previously been successfully tested in other grassland-drought experiments (Hofer *et al.*, 2016, 2017). Controls

Table 1: Overview of the timing of the seasonal treatments in 2014 and 2015.

	Spring treatment	Summer treatment	Fall treatment
2014			
Drought duration	03-12 – 05-21	05-21 – 07-30	07-30 – 10-17
Harvests			
During drought	05-21	7-30	10-17
Post-drought	07-30	10-17	05-20 (in 2015)
2015			
Drought duration	03-11 – 05-20	05-20 – 07-29	07-29 – 10-07
Harvests			
During drought	05-20	07-29	10-07
Post-drought	07-29	10-07	----------

were subject to the natural precipitation regime. However, when soil water potential (Ψ_{Soil}) sank below -0.5 MPa due to naturally dry conditions, control plots were additionally watered with 20 mm of water (300 l per plot). Watering happened once on June 16th and 17th 2014 and three times in 2015 (7./8.7., 14./15.7., 11./12.8.).

Environmental measurements

Air temperature and relative humidity were measured at the field site using VP-3 humidity, temperature and vapor pressure sensors (Decagon Devices, Inc., Pullman, WA, USA) at an hourly interval. Measurements were conducted under control and treatment conditions (n=2). Information on precipitation and evapotranspiration was provided by the national meteorological service stations that were in close proximity of the research site (average of the two surrounding meteorological stations Zurich Affoltern in 1.4 km distance and Zurich Kloten in 4.5 km distance). Ψ_{Soil} was measured on an hourly basis using 32 MPS-2 dielectric water potential sensors (Decagon Devices, Inc., Pullman, WA, USA) that were installed in 10 cm depth. The soil water potential sensors were evenly distributed over the field site and treatments. Daily means of all measurements were calculated per treatment, but across species since no species-specific alterations in Ψ_{Soil} were expected (Hoekstra *et al.*, 2014) or measured (n=8).

Harvests

Aboveground biomass was harvested six times per year at a five-week interval in 2014 and 2015 (see Table 1, Fig. 1). The harvests occurred five and ten weeks after the installation of the drought treatments on the respective treatment. The aboveground biomass was cut at 7 cm height from a central strip of each plot (5 × 1.5 m) using an experimental plot harvester (Hege 212, Wintersteiger AG, Ried/I., Austria). Dry matter yield of each plot was determined by weighing and drying a biomass subsample at 60°C for 48 h.

Forage quality measurements

For quality analysis the dried biomass subsamples of the harvested plant material were ground using a cutting mill (Schneidmühle SM200, Retsch, Germany) to pass through a 0.75 mm sieve. The milled plant material was analyzed using near-infrared reflectance spectrometry (NIRS) with a dispersive infrared spectrometer (NIR model 6500, FOSS;

Hilleroed, Denmark) and validated by *in vitro* analysis. The NIRS technique is based on the relationship between the reflectance spectrum of light passing through the sample and the wavelength regions that are associated with certain chemical constituents within the sample. Via regression relationships between NIRS spectral data and *in vitro* laboratory or *in vivo* measurements forage quality parameters can be predicted. The advantage over *in vitro* and *in vivo* analyses is that the NIRS technique requires no chemical reagents and offers non-destructive, fast and accurate forage evaluation (Adesoganl *et al.*, 2000). For this study the forage samples were analyzed for nitrogen (N) and crude ash (CA) and on the basis of standard calculations (Agroscope, 2017) digestible organic matter (DOM) and net energy of lactation (NEL) were estimated.

The nitrogen nutrition index (NNI) as a parameter to define the N status of a plant was calculated as follows:

$$NNI = N/N_c$$

N is the measured total N concentration of the harvested plant material and N_c is the critical total N concentration on basis of the dry matter yield (DMY). N_c is the minimum N concentration needed to achieve maximum DMY by the plant and is calculated according to Lemaire (1997):

$$N_c = 4.8 \times DMY^{-0.32}$$

NNI values equal or above 1 indicate non-limiting N supply, while values below 1 indicate N deficiency at the time of growth.

Data analysis

Changes in the forage quality parameters nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) were tested similarly for impacts of drought during the drought treatment and for impacts of drought legacy after ten weeks of the post-drought period. Changes in forage quality were analyzed by one-way analysis of variance. The first factor season consisted of three levels (spring, summer, fall), the second factor treatment consisted of two factors (control, drought treatment) and the last factor consisted of the factor year with the two levels 2014 and 2015.

Results

Forage quality parameters in the grasses and the legume species differed throughout the growing season (Fig. 2). In grasses, N content was increasing from the beginning of the growing season towards the end in both years and N content was higher in 2015 than in 2014 (Table 2). In the legume, N content was generally higher than in the grasses in both years. Moreover, N content was highest at the end of each year in harvest six, like in the grasses, but lowest in the middle of each year during harvest four (Fig. 2). Crude ash (CA) content did not show any distinct pattern throughout the growing seasons for grasses. In both years 2014 and 2015, the amount of CA in grasses laid between 90 and 100 g/kg dry matter. In the legume species, CA content was higher in 2014 than in 2015 and also higher than in the grasses, lying between 110 and 120 g/kg dry matter. In 2015, CA content in the legume species was increasing towards the end of the growing

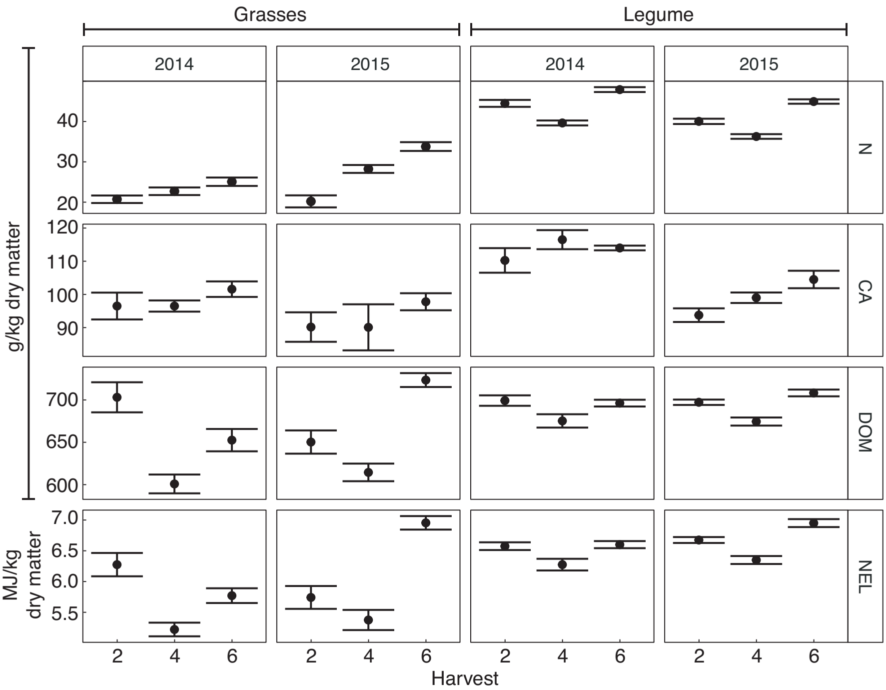

Fig. 2: The contents of nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) in plant material harvested under control conditions during the harvest two, four and six throughout the growing seasons 2014 and 2015. Values are the mean of the investigated grasses (left; n=6, ± se) and the legume species *T. repens* (right; n=4, ± se).

season, starting with a content of 94 g/kg dry matter at harvest two and resulting in a CA content of 105 g/kg dry matter at harvest six. Digestible organic matter (DOM) showed a much wider spectrum of variation in grasses than in the legume in both years. While DOM in grasses was at levels around 600 g/kg dry matter in the fourth harvest of both years, DOM in the legume was also lowest during the fourth harvest, but at levels of around 675 g/kg dry matter. In grasses, highest levels of DOM could be found during the second harvest of 2014 and the sixth harvest of 2015. This was similar for

Table 2: Summary of analysis of variance for the effects season, drought treatment, year, and their interactions on the fodder quality parameters nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) at the end of the seasonal drought periods for grasses.

		N		CA		DOM		NEL	
Effect	df	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Season (spring, summer, fall)	2	63.4	<0.001	4.4	0.016	37.1	<0.001	43.8	<0.001
Treatment (control vs. drought)	1	0.6	0.459	11.8	0.001	0.3	0.577	0.7	0.395
Year	1	30.2	<0.001	6.3	0.015	0.4	0.509	3.4	0.071
Season × Treatment	2	0.3	0.778	0.3	0.708	0.2	0.816	0.1	0.880
Season × Year	2	6.6	0.003	1.7	0.200	23.9	<0.001	32.0	<0.001
Treatment × Year	1	1.4	0.246	0.1	0.769	0.2	0.680	0.6	0.429
Season × Treatment × Year	2	2.4	0.102	2.6	0.083	0.5	0.636	0.2	0.786
R^2		0.71		0.27		0.62		0.68	

the legume species, but with lower differences between the DOM content of the different harvests. The pattern for NEL was similar to that of DOM for both, grasses and legumes, in both years.

In general, the changes in forage quality due to seasonal drought differed between grasses and the legume species. In the grasses the changes due to drought were less pronounced than in the legume species. Although increased N contents could be found under summer and fall drought in 2014 and under spring drought in 2015 (Fig. 3), N content was not significantly affected by drought (Table 2; treatment p=n.s.) and did not increase with increasing drought intensity (Fig. 4). CA contents were significantly reduced by drought (Table 2; treatment $p<0.005$) and could especially be detected

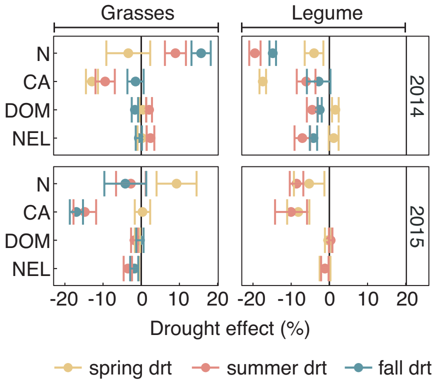

Fig. 3: The mean effect of drought during spring, summer and fall on the forage quality variables: nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) of the years 2014 and 2015. Values are the mean of the investigated grasses (left; n=6, ± se) and the legume species *T. repens* (right, n=4, ± se). Forage quality parameters that are reduced under drought are located left from the vertical black line, increased parameters are located right from the line.

under spring and summer drought in 2014 and under summer and fall drought in 2015, but the reduction of CA under drought did not depend on the drought intensity (Fig. 4). DOM and NEL in the grasses were not altered (less than 5%) under seasonal drought in both years (Fig. 3, Table 2; treatment p=n.s.), and thus, showed values similar to those of the control plants with decreasing Ψ_{soil} (Fig. 4). Drought in the legume species mainly lead to reductions in the forage quality parameters. N and CA content were reduced under every seasonal drought in both years (Fig. 3, Table 3; treatment $p<0.001$). The reduction of N content even increased with increasing drought intensity (= decreasing Ψ_{soil}), while CA content was similarly low independent from drought intensity (Fig. 4). For the legume species, DOM and NEL were reduced under summer and fall drought in 2014, but not under spring drought (Fig. 3, Table 3; season × treatment $p<0.05$).

When compared to corresponding controls, grasses showed an increase of N content after drought release in the post-drought period in every season (Fig. 5, Table 4; treatment $p<0.001$). With reductions in Ψ_{soil} the N content was not reduced in the post-

Table 3: Summary of analysis of variance for the effects season, drought treatment, year, and their interactions on the fodder quality parameters nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) at the end of the seasonal drought periods for the legume.

		N		CA		DOM		NEL	
Effect	df	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Season (spring, summer, fall)	2	131.4	<0.001	26.1	<0.001	39.9	<0.001	40.6	<0.001
Treatment (control vs. drought)	1	77.5	<0.001	23.6	<0.001	8.2	0.007	19.2	<0.001
Year	1	28.1	<0.001	75.5	<0.001	4.0	0.052	18.1	<0.001
Season × Treatment	2	9.2	<0.001	1.9	0.162	5.0	0.013	4.8	0.015
Season × Year	2	5.4	0.009	3.1	0.057	5.5	0.009	3.7	0.037
Treatment × Year	1	3.8	0.060	1.3	0.254	1.6	0.209	1.0	0.319
Season × Treatment × Year	2	5.2	0.029	3.6	0.067	9.0	0.005	5.6	0.024
R^2		0.90		0.78		0.73		0.75	

drought period (Fig. 6). Legumes, in contrast, did not only not show an increase of N in their plant tissue, in the year 2015 the N content even decreased in the post-drought period (Fig. 5, Table 4; treatment $p<0.001$). Decreased N contents in the legume are particularly prevalent under lower Ψ_{soil}, but the relationship was not linear (Fig. 6). All other forage quality parameters did not show any legacy effects to seasonal drought periods post-drought (Fig. 5, 6, Table 4, 5; treatment p=n.s.).

Although nitrogen nutrition index (NNI) of the grasses was always below 1, under control conditions as well as under drought (Fig. 7), values of NNI were always lower for grasses under drought than for the corresponding control plants in both years

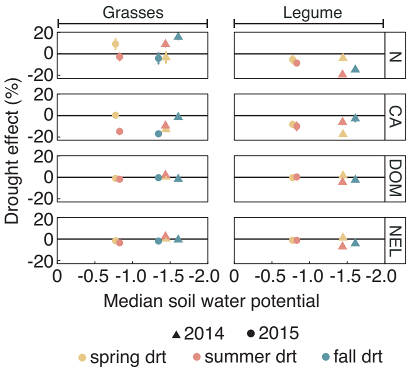

Fig. 4: The mean effect of drought with increasing drought intensity (expressed as the median of the soil water potential (Ψ_{soil})) on the forage quality variables: nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL). Values are the mean of the investigated grasses (left; n=6, ± se) and the legume species *T. repens* (right, n=4, ± se) per year. Forage quality parameters that are reduced under drought are located below the horizontal black line, increased parameters are located above the line.

(except under drought in spring 2014). NNI for the legume species was above 1 in the beginning of both growing seasons 2014 and 2015 (harvest 2), for the control plants as well as for the plants under drought (Fig. 7). Towards the end of the growing seasons NNI of the legume species decreased below values of 1 and values under drought conditions were always lower than values of the corresponding control plants (Fig. 7).

For grasses, NNI after drought release was always below 1 and did not differ between control und previously drought stressed plants in both years (Fig. 7). The values of NNI for the legume in the post-drought period were above 1 at the beginning of the growing season (harvest 2), similar to during the drought periods. Later in the growing season the NNI values for the legume decreased to values under 1, but were similar for control plants and previously drought stressed plants (Fig. 7).

Table 4: Summary of analysis of variance for the effects season, drought treatment, year, and their interactions on the fodder quality parameters nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) after ten weeks of the post-drought period for grasses.

		N		CA		DOM		NEL	
Effect	df	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*P*
Season (spring, summer, fall)	2	60.5	<0.001	4.2	0.020	58.2	<0.001	75.9	<0.001
Treatment (control vs. drought)	1	20.5	<0.001	0.1	0.713	0.0	0.888	0.7	0.370
Year	1	98.5	<0.001	4.1	0.049	37.7	<0.001	3.4	<0.001
Season × Treatment	2	2.4	0.105	0.2	0.819	0.1	0.940	0.1	0.657
Season × Year	1	7.0	0.011	0.2	0.621	24.5	<0.001	46.6	<0.001
Treatment × Year	1	2.1	0.152	0.2	0.625	0.3	0.562	0.6	0.378
Season × Treatment × Year	1	0.6	0.453	0.0	0.916	1.4	0.243	0.2	0.210
R^2		0.81		0.07		0.74		0.81	

Discussion

Testing the effects of drought on forage quality showed strong differences between grasses and the legume species. While N content, DOM and NEL of grasses were not affected by drought, forage quality (determined by high values of N, DOM and NEL) was reduced under drought in the legume, but did not show any relationship to drought intensity. Additionally, drought-release resulted in a forage quality increase due to a higher N content in the grass plant tissue in the post-drought period, while the nutritive value of the legume tissue stayed unaffected. However, differences in the forage quality

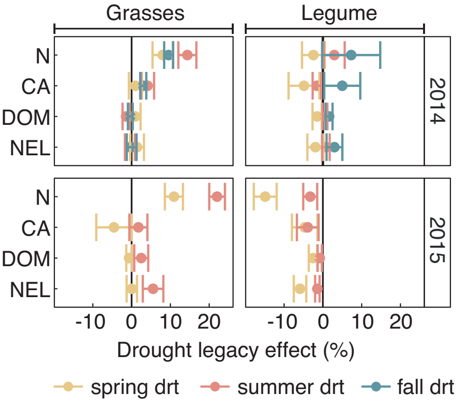

Fig. 5: The mean effect of drought legacy after spring, summer and fall drought on the forage quality variables: nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) of the years 2014 and 2015. Values for fall resilience are values from the first growth period of the following year 2015. Values are the mean of the investigated grasses (left; n=6, ± se) and the legume species *T. repens* (right, n=4, ± se). Forage quality parameters that are reduced post-drought are located left from the vertical black line, increased parameters are located right from the line.

between the seasons were detectable for both, grasses and the legume, highlighting the seasonality of grasslands not only with regards to productivity.

The tested forage quality parameters varied more or less throughout the growing season. N and CA content increased in the course of one year in grasses and the legume. Such clear increases in nutrients were not expected since Smith *et al.* (1998) showed decreasing N values with increasing days after anthesis, ending in constant values. DOM and NEL showed mostly variations between the years, especially in grasses. In *Lolium* species, which were also included in our analyses, DOM has been shown to decrease with the number of regrowths (Schubiger *et al.*, 1997; Smith *et al.*, 1998), Since variations in our experiment were high, an overall decrease with increasing regrowths could not be confirmed. Higher values of DOM in the legume compared to the grasses, which we found in our study, is an already well-documented feature (Grant *et al.*, 2014).

Increases in N content under drought have been detected in a majority of experiments, but with high variations among experiments leading to an average increa-

Table 5: Summary of analysis of variance for the effects season, drought treatment, year, and their interactions on the fodder quality parameters nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL) after ten weeks of the post-drought period for the legume.

		N		CA		DOM		NEL	
Effect	df	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	p
Season (spring, summer, fall)	2	83.0	<0.001	18.1	<0.001	45.3	<0.001	53.7	<0.001
Treatment (control vs. drought)	1	1.1	0.297	2.9	0.099	1.1	0.307	1.6	0.211
Year	1	41.2	<0.001	73.5	<0.001	0.1	0.705	3.4	0.076
Season × Treatment	2	5.2	0.012	3.2	0.056	3.6	0.041	4.7	0.016
Season × Year	1	0.6	0.440	3.6	0.067	2.1	0.154	7.0	0.013
Treatment × Year	1	5.6	0.025	0.0	0.847	1.1	0.302	2.7	0.113
Season × Treatment × Year	1	0.3	0.603	0.3	0.565	0.0	0.875	0.2	0.686
R^2		0.85		0.75		0.70		0.76	

se in N by 5% (Dumont *et al.*, 2015). In our study, the variations in the grasses were, indeed, high under drought reaching from no changes in the N content in the plant tissue to increases by more than 15% under drought in fall 2015. Surprisingly, the nutrient supply in the legume was decreased under drought, although legumes are able to assimilate atmospheric N_2 and should not be restricted to a water-soluble N supply under drought conditions. However, symbiotic N fixation of legumes is very sensitive to drought, most probably due to reduced turgor pressure, which affects the nodule activity (Serraj *et al.*, 1999; González *et al.*, 2015). Reduced N content in the legume plant material, thus, can be explained by limited N supply due to decreasing N fixation rates.

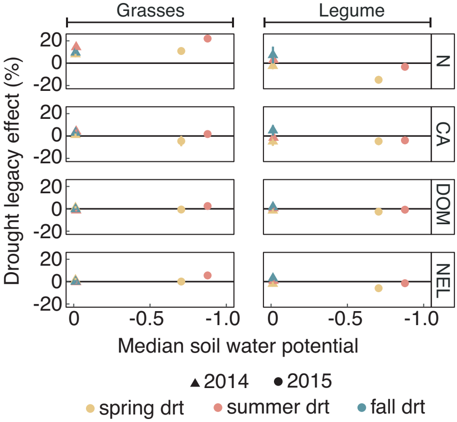

Fig. 6: The mean effect of drought legacy with increasing drought intensity (expressed as the median of the soil water potential (Ψ_{soil})) on the forage quality variables: nitrogen (N), crude ash (CA), digestible organic matter (DOM) and net energy of lactation (NEL). Values for fall resilience are values from the first growth period of the following year 2015. Values are the mean of the investigated grasses (left; n=6, ± se) and the legume species *T. repens* (right, n=4, ± se) per year. Forage quality parameters that are reduced post-drought are located below the horizontal black line, increased parameters are located above the line.

Nonetheless, NNI shows that the legume was less restricted in N supply throughout the experiment than the grasses.

A meta-analysis by Dumont *et al.* (2015) found DOM increases with drought by about 7%. In our study, we could not find any drought effects on DOM in grasses, but DOM was decreasing in the legume species under drought. Since Dumont *et al.* (2015) found strong experimental variations leading to this average increase of DOM, the absent or negative effects of drought on DOM in our study are not to be overstated. As for DOM, NEL for grasses was not affected by drought, but reduced for the legume. Although literature on NEL under drought is scarce, Grant *et al.* (2014), who tested precipitation variability on NEL, also found no effect of high variability on NEL in grasses.

In contrast to Küchenmeister *et al.* (2014), we found increases in nutritive value after drought release in grasses. Possibly, increases in soil water after drought-release in the post-drought period lead to increases in the soil N flux (Birch, 1964). A variety of

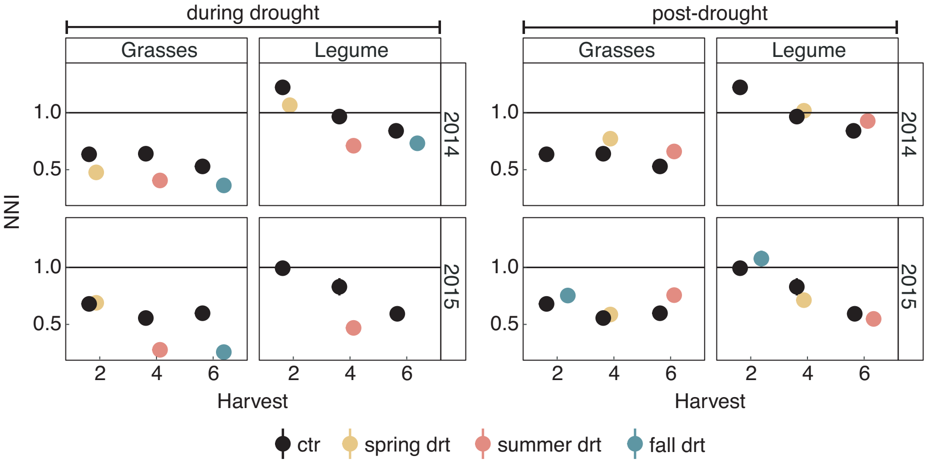

Fig. 7: Nitrogen nutrition index (NNI) of harvested plant material from control and drought treatment plants (left) and from control and from drought-released plants in the post-drought period (right). Values for fall resilience are values from the first growth period of the following year 2015. Values are the mean of the investigated grasses (left; n=6, ±se) and the legume species *T. repens* (right, n=4, ±se) per year. Values below the black horizontal line indicate limited nitrogen supply, values above the line indicate sufficient nitrogen maintenance.

mechanisms can explain these N increases in rewetted soil, like accumulated microbial and plant necromass or the lysis of living microbial cells (Borken & Matzner, 2009), which can be responsible for N increases in plant tissues post-drought.

Although seasonal differences in forage quality emerged and reactions of forage quality parameters to drought could be detected, a consistent sensitivity of certain forage quality parameters to drought events in a specific season could not be identified. The alterations of forage quality were variable, depending on the investigated parameter and also on the plant functional type investigated. However, disentangling the effects of drought and the timing of a drought event on forage quality should be improved, maybe by testing different plant developmental stages that reflect the seasonality of temperate grasslands more precisely.

Acknowledgements

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Concluding discussion

Increasingly arising drought events caused by climate change are known to affect grassland ecosystems all over the world (Chu *et al.*, 2016). Plant community reactions to drought are well-investigated (Wu *et al.*, 2011), but studies so far have disregarded the seasonal character of grasslands. Different stages of productivity, morphology, ontogeny, phenology or physiology that grasses undergo during their life cycle, may lead to different sensitivities of grasslands depending on the stage the plants are in during a drought event. The focus of the present study, hence, was to investigate seasonal effects of droughts on grassland ecosystems. Comparing impacts of drought between different seasons is quite challenging since defined and analogous levels of stress are difficult to achieve. Installing drought treatments that equal in their duration does not mean they automatically equal in their drought intensity. However, determining the right response variable and considering the drought stress on a continuous scale allows the comparison of possibly differing drought intensities. Doing this allowed us to address different scopes. For one thing, the study was designed to understand the ecosystem responses und underlying mechanisms of grasslands due to drought events that occur in different times of the growing season. The *chapters 1* and *2* approach this area with investigating growth and physiological responses to seasonal droughts. Furthermore, with examining differences in forage quality, *chapter 3* comprises an applied scope of seasonal drought events on the grassland ecosystem.

Growth responses due to drought have been investigated in a variety of experiments and studies are consistent regarding the negative implications of drought events (Wu *et al.*, 2011). Supporting that, in this study growth reductions were detected regardless of when in the year a drought event occurred. However, growth during the most productive season (spring) was most resistant to drought. Higher growth rates of plants under drought in spring can be attributed to a higher physiological activity (assessed by Ψ_{Leaf}, g_s and $d^{13}C$) during drought in spring compared to the other seasons. Since grasses were in reproductive stages mainly in spring, maintaining physiologically active especially during spring drought leads to one assumption: To ensure reproduction and, thus, the survival of the community, the grasses invest in stress resistance mechanisms under drought in spring compared to seasons of vegetative growth. For example, osmotic adjustment (OA) increases the osmotic potential by accumulation of solutes within the plant cells, hence, maintaining important metabolic processes while under drought (Sánchez *et al.*, 1998). An investment in OA under drought especially during critical reproductive growth stages in spring is beneficial for grasses and is reflected in the higher resistance of spring growth under drought compared to other seasons.

Besides immediate plant responses to drought, several studies have revealed negative legacy effects on the productivity of ecosystems after the release from drought (Sala *et al.*, 2012; Reichmann *et al.*, 2013). In this study though, distinct positive legacy effects were observed. Growth rates of previously drought-stressed plots were larger than growth rates of corresponding control plots with no drought-history, indicating a high resilience of the stressed plants. The drought legacy even induced positive growth responses in the beginning of the following growing season. The precise mechanisms for this positive legacy effects are unclear, but according to Gordon *et al.* (2008) increases in microbial activity after rewetting of the soil can be the reason for an influx of plant available nutrients in the soil. This is supported by Hofer *et al.* (2017) who also found increasing N availability in the soil in the post-drought period. Besides nutritional mechanisms of positive legacy effects, also intrinsic processes are shown to be triggered after rewetting of formerly drought-stressed plants (Arend *et al.*, 2016; Hagedorn *et al.*, 2016).

Immediate negative growth responses under drought and positive legacy effects after drought-release are offsetting each other largely. Cumulative biomass at the end of one growing season is hardly negatively affected by drought, because growth losses under drought are compensated by growth increased after drought, resulting in a productivity comparable to those of non-stressed plants. However, one must consider the seasonality of grasslands. Although seasons of high productivity (e.g. spring) are less sensitive to drought, a drought event still has the potential to result in high absolute growth losses. On the other hand, increased post-drought growth rates can be relatively high and still result in a compensation close to zero when absolute growth is at its minimum (e.g. in fall). Therefore, the timing of a drought event still plays an important role.

Nutrient concentration is known to naturally differ along the growing season, altering the forage quality of grasses (Schubiger *et al.*, 1997; Smith *et al.*, 1998). Whether or in which direction drought events influence the nutrition and quality of forage is not fully solved, because studies are contradicting (N increase under drought: Grant *et al.* (2014); Dumont *et al.* (2015); no effect of drought on N: Küchenmeister *et al.* (2014)). Beyond that, seasonal differences of drought events on forage quality have been unfamiliar territory until this study.

While forage quality (here defined as contents of N, digestible organic matter (DOM) and net energy of lactation (NEL)) of grasses differed between seasons, it was not affected by drought events in either season. In contrast, the tested legume species not only showed alterations in its nutritional value between the seasons. Moreover, the forage quality in the different seasons was differently affected by drought events (quality of summer forage more negatively affected than the quality of fall or spring forage). Although the quality of the grasses was not reduced under drought, the post-drought period following a drought event, nevertheless, achieved an increase of N regardless of the season. The forage quality of the drought-affected legume, in contrast, remained unaffected after drought-release.

While drought in general did not affect the nutrition of grasses directly, but rather increased N content after drought-release, seasonal effects of drought were evidenced by legumes, reducing forage quality of at least *T. repens* L. The partial resistance of forage quality to seasonal droughts is of importance for applied sciences, such as the agricultural sector. Beyond that, the productivity of grasses being differently sensitive to drought events depending on the season a drought occurs and also the partial robustness of annual productivity of grasses is advantageous knowledge for stakeholders from the agricultural industry (e.g. farmers, seed industry, agricultural schools).

Consequently, understanding seasonal impacts of drought, especially on the productivity of grasses, is of high importance for a variety of areas. Besides delivering applied improvements comprehensive knowledge of one of the main ecosystems worldwide is achieved. Understanding seasonal differences and seasonal processes of grasslands is a key factor for understanding the global C cycle. Especially with regards to climate change, detailed and precise knowledge of grassland functioning supports the enhancement of climate change models and the understanding of ecosystem responses (Jentsch *et al.*, 2007; Leuzinger *et al.*, 2011).

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